JOURNAL OF AGRICULTURAL RESEARCH

VOL. X WASHINGTON, D. C., JULY 23, 1917 NO. 4

A COLLETOTRICHUM LEAFSPOT OF TURNIPS

By B. B. HIGGINS,
Botanist, Georgia Agricultural Experiment Station

DESCRIPTION OF THE LEAFSPOT

During August, 1914, young turnip plants (*Brassica rapa*) with leaves badly affected by a leafspot disease were brought to this Station from near Macon, Ga. The grower stated that during two previous seasons the disease had been very destructive to young turnips, but observations on specimens sent from this field indicate that the greater part of damage was probably due to a bacterial softrot of the roots. Since that time the diseased plants have been collected locally several times. The disease rarely killed the plants or proved very serious, other than in disfiguring and occasionally killing the tops.

The spots are small (¼ inch or less in diameter), circular in outline, and of a pale-gray or straw color. Usually no sign of a parasite can be seen with the unaided eye or even with a good hand lens; but, if the spot has developed under very moist conditions, both surfaces are covered with a salmon-pink layer of spores, a condition which is evident to the unaided eye. Under the microscope this layer is found to be composed of small, rod-shaped, 1-celled spores which singly appear colorless. They are borne on short sporophores which arise in small clusters from a delicate stroma in the surface of the leaf. Intermixed with these sporophores are prominent, long, black setæ, indicating at once the affinity of the fungus to the genus Colletotrichum.

Cultures of the fungus were readily obtained by sowing the spores in an agar plate. The mycelial growth was rather inconspicuous on all media, much more so than that from a culture of *Glomerella cingulata* from a decaying pear. Green bean pods, steamed in an autoclave and inoculated with the fungus from turnips, were covered in a few days with a thick layer of salmon-colored spores. On corn-meal agar [1] the acervuli, instead of being scattered evenly over the surface as on bean pods, were grouped in clusters. In old cultures on various media black structures having the appearance of perithecia were produced in abundance; but, when examined under the microscope, these were found to be only dense clusters of setæ. No perithecia were ever found either in cultures or on the infected leaves.

[1] SHEAR C. L., and WOOD, Anna K. STUDIES OF FUNGUS PARASITES BELONGING TO THE GENUS GLOMERELLA. U. S. Dept. Agr. Bur. Plant Indus. Bul. 252, p. 15. 1913.

INOCULATION EXPERIMENTS

Seedlings of the Purple Top variety of turnips, which had been developed in pots in the greenhouse, were sprayed with a suspension of the fungus spores in water. For this purpose a bean-pod culture, covered with the pink spore mass, was shaken in sterilized water. The suspension was then poured into a sterile atomizer used for spraying. At the same time similar seedlings were sprayed with sterile water, and both lots were covered with bell jars during the first four days. On the fourth day dark water-soaked spots began to appear on the plants sprayed with the spore suspension. The spots soon became very numerous and faded to the light-straw color characteristic of naturally infected spots. The control plants, sprayed with sterile water, remained free from the disease.

On March 15 several plants of mixed varieties from seed sown in the field the previous autumn were transferred to pots and carried into the greenhouse. After the plants had recovered from the transplanting and had begun to grow, they were inoculated as described above. Abundant infection was obtained on all varieties, indicating that probably no varieties of turnips are immune. Not only the leaves but also the stems and seed pods showed infection. Some of the very young seed stalks were killed, the whole outer surface being covered with anastomosed spots.

Inoculations were also made in a similar manner on radish (*Raphanus sativus*), cabbage (*Brassica oleracea capitata*), collard (*Brassica oleracea viridis*), and lettuce (*Lactuca sativa*). The inoculations on radish plants gave abundant infection. The radish seemed to be fully as susceptible as the turnip. On cabbage a few spots developed, but not nearly so abundantly as on turnip and on radish. Collards seemed to be slightly more susceptible than cabbage, but no naturally infected plants of either have ever been found in the field. The lettuce plants showed no sign of infection, though inoculated on various occasions.

A few cross-inoculations have been made in studying the relation of this fungus to other allied forms. Several turnip plants in each of five pots were inoculated as follows: No. 1 with the Colletotrichum from turnip, No. 2 with conidia of *Glomerella cingulata* from pear, and No. 3 with conidia of *Glomerella gossypii* from cotton bolls. The two others were not inoculated, but were given otherwise similar treatment to serve as control plants. On the fifth day spots began to appear on the plants of pot 1 sprayed with the Colletotrichum from turnip, and after a few days infection became so abundant that all the leaves were killed on these plants. No spots ever occurred either on the control plants or on the plants of pots 2 and 3.

At the same time three sound bean pods were sprayed with a water suspension of conidia of the Colletotrichum from turnip, five others were likewise sprayed, after being pricked or scratched with a sterile needle, and similar numbers to serve as controls were sprayed with sterile

water. All were inclosed in flasks for four days. No infections occurred, the wounds on both inoculated and uninoculated pods healing normally.

Six sound, firm apples were washed with a 1-to-500 solution of mercuric chlorid, rinsed in sterile water, and placed three in each of two sterile culture dishes. Three were punctured with a sterile scalpel and inoculated with conidia and mycelium from a culture of the turnip fungus; and the three others were punctured in a similar manner and inoculated with conidia and mycelium from a culture of *Glomerella cingulata* from pear. All were left at room temperature in the laboratory. After a month one of those inoculated with *G. cingulata* was almost entirely decayed, and each of the others so inoculated showed spots an inch or more in diameter, while those inoculated with the species of Colletotrichum from turnip showed only slight decay immediately within the wound. Later, as the fruits began to ripen, the decay spread until the whole of each was involved.

SEED INFECTION

As previously mentioned, inoculations showed that the seed pods were readily and abundantly infected. In view of the fact that it has been shown that under similar conditions the mycelium of some other species of this genus, notably *Glomerella* (*Colletotrichum*) *lindemuthianum* Shear and *G.* (*Colletotrichum*) *gossypii* Edgerton, enters the seed and remains dormant there during dormancy of the seed, this observation was of special interest; and experiments were planned to determine whether this was true for the turnip fungus.

During the spring of 1915 several old turnip plants in the field were transferred to pots and brought into the greenhouse to produce seed. When seed pods of various ages, from flowers to almost mature pods, had formed on a stalk the whole seed stalk was sprayed with a suspension of spores in water and then covered for a few days with a glass cylinder. Within a short time practically every pod on the inoculated plants showed one or more spots, and on many the entire surface was diseased. When the seed had ripened, the stalks were cut and hung in the storeroom until the experiment could be completed.

At various times during the following fall and winter seed were carefully shelled out, counted, and planted in pots in the greenhouse. Many seed from badly infested pods were plainly shrunken and dead. Less than half germinated. In one lot of 500 seed planted only 14 germinated. In all more than 2,000 seed were planted; but in no case could any diseased spots be found on the cotyledons or hypocotyl of the seedlings and none developed on the leaves.

Some of the diseased seed pods were also killed, embedded in paraffin, cut, and stained with various stains; but no trace of mycelium was ever found in living seed. It seems that, when the fungus enters a seed, the young embryo is killed at once, and the fungus probably dries up and

dies along with the seed. This is indicated by the macroscopical and histological appearance of the seed, and also by the results of the germination tests, in so far as the negative results can be held to justify a conclusion. It might readily be expected to happen in seed so small as those of the turnip.

IDENTITY OF THE FUNGUS

The form genera Colletotrichum and Gloesporium, comprising the group to which this organism belongs, are very difficult to separate into species because of the fact that many species are morphologically very variable and are rather general parasites—that is, a single species may infect and produce some form of disease in a great variety of host plants, as shown by the work of Shear and Wood [1] and others. Therefore one is necessarily more or less uncertain as to just how much reliance should be placed on morphological structures or on inoculation tests in determining the limits of species.

The results of cross-inoculations indicate that the turnip fungus is probably not identical with either *Glomerella lindemuthianum*, *G. gossypii*, or *G. cingulata*. The last-named species, however, according to the views of Shear and Wood,[1] contains several forms which are more or less distinct both morphologically and phisiologically. Therefore, if this view is accepted, it is impossible to determine the limits of this species in the absence of the ascigerous stage.

Two related forms, *Gloeosporium concentricum* (Grev.) Berk. and Br.[2] and *Colletotrichum brassicae* Schulz and Sacc.,[3] have been described on species of Brassica. Unfortunately specimens of neither of these could be obtained for comparison; but specimens of the turnip fungus were sent to Dr. C. L. Shear, of the Bureau of Plant Industry, who compared it with *G. concentricum*, which, he says, is entirely distinct. He expressed the opinion, however, that the turnip fungus might be identical with *C. brassicae*, specimens of which were not available to him. This species was described as occurring on decaying stems of *Brassica oleracea* and *B. caulocarpa;* but the characterization was very brief and, in view of the recent work in this group, is of little value in determining relationships.

The fungus and the disease produced on turnip leaves is characterized as follows: Spots small, circular, or nearly so, 2 to 5 mm. in diameter, sometimes anastomosing and forming larger spots of irregular shape, pale grayish or straw colored; acervuli small, scattered over both lower and upper surface of spot; stromata subepidermal, delicate; conidiophore short, tapering abruptly to a slender sterigmata to which the spore is attached; conidia cylindrical, 13.5 to 19.5 by 4 to 5.5 μ, hyalin, 1-celled; setæ dark brown to black, slender, 20 to 42 by 3 to 5 μ, 1- to 3-septate.

[1] SHEAR, C. L., and WOOD, Anna K. Op. cit.

[2] GREVILLE, R. K. SCOTTISH CRYPTOGAMIC FLORA. v. 1, pl. 27 (col.). Edinburgh, 1823.

[3] SCHULZER VON MÜGGENBURG, Stephan, and SACCARDO, P. A. MICROMYCETES SCLAVONICI NOVI. *In* Rev. Mycol., ann. 6, no. 22, p. 79. 1884.

On the stems the spots are more elongated parallel with the long axis of the stem.

The fungus seems to differ morphologically from *Colletotrichum brassicae* Schulz. and Sacc., especially as to shape and size of spores (Pl. 13, *A*; 14). (The spores of *C. brassicae* are described as fusoid, slightly curved, and 19 to 24 μ in length.) But it seems best to refer it tentatively to this species until a careful comparison of the two forms can be made.[1]

SUMMARY

An apparently new leafspot of turnips has been found in various localities of Georgia.

The disease also attacks the stems and seed pods, but experiments indicate that the fungus is not carried over in the living seed.

The fungus causing the disease is tentatively referred to *Colletotrichum brassicae* Schulz. and Sacc.

The spots are much smaller than the similarly shaped spots produced by *Cylindrosporium brassicae* Fautr. and Roum., which has been very abundant in this region during the past two years (Pl. 13, *B*). Both organisms are frequently found on the same plant.

[1] Specimens of this fungus were submitted to Dr. P. A. Saccardo, who finds that the fungus is not identical with *Colletotrichum brassicae*. He considers it a new species, to which he gives, in a note received after this article had been forwarded for publication, the following name and diagnosis:

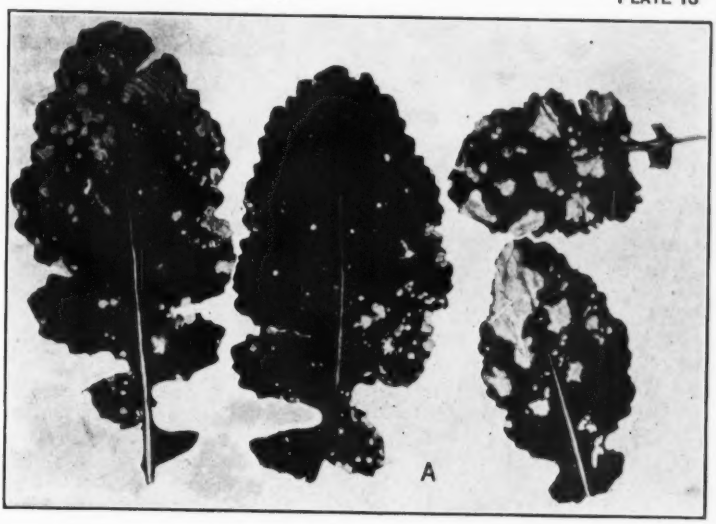
Colletotrichum Higginscanum, species novæ. Maculis amphigenis, crebris, subrotundis, 2–4 mm. diam. pallide alutaceis, marginulo elevato, angusto virescenti-cinctis; acervulis punctiformibus, aegre visibilibus; setulis parcis filiformibus, fuligineis, sursum pallidioribus, 1–2-septatis 45–70=3–6, rectusculis; conidiis terete-fusoideis, 15–17=3–33, utrique obtusatis, pluriguttulatis, rectis, hyalinis; conidiophoris paliformibus, parce septatis, 16–19=6, subhyalinis.

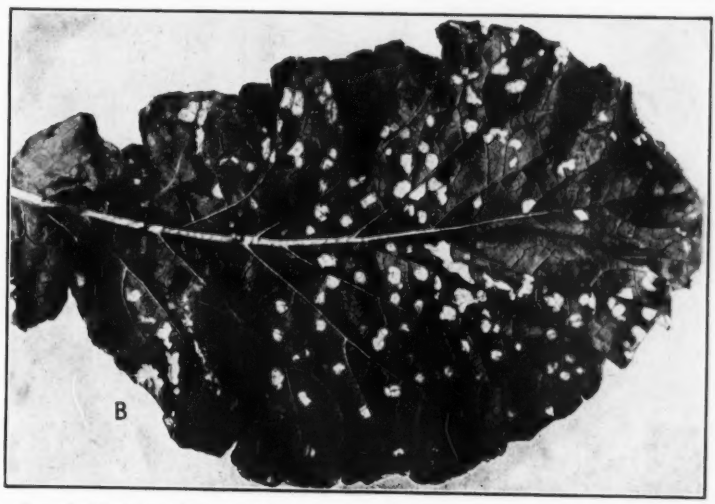
Hab. in foliis adhuc vivis *Brassicae Rapae*, Georgia, Am. bor. A *Coll. Brassicae* recedit, maculis, situ, conidiis non curvis, etc.

PLATE 13

A.—Leaves of a turnip nine days after inoculation with conidia of *Colletotrichum brassicae*. Slightly reduced.

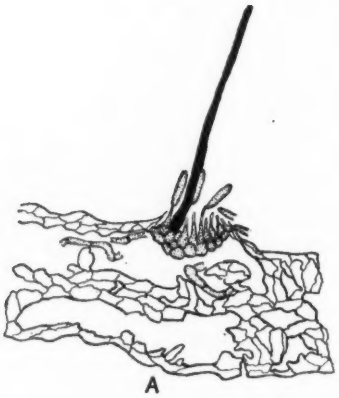
B.—A turnip leaf showing spots produced by *Cylindrosporium brassicae*. Slightly reduced.


A


B

A Colletotrichum Leafspot of Turnips

PLATE 14



A

B

PLATE 14

Colletotrichum brassicae from turnip:

A.—Section through an acervulus on a turnip leaf. × 364.
B.—Conidia from a turnip leaf. × 728.

BLACK ROOTROT OF THE APPLE[1]

By F. D. FROMME, *Plant Pathologist*, and H. E. THOMAS,[2] *formerly Assistant Plant Pathologist, Virginia Agricultural Experiment Station*

INTRODUCTION

An unusually destructive rootrot of the apple (*Malus sylvestris*) is prevalent in the chief orchard sections of Virginia. The disease has been known to the growers for a number of years, but it is only quite recently that considerable interest has been manifested in it and a technical study undertaken at the Virginia Experiment Station.

The first published record of the disease, by Reed and Crabill (10) in 1913, contains a brief mention of a rootrot, with the suggestion that the cause may be one or more mushrooms. Subsequent preliminary notes by the same authors (11) and by Crabill (1) have outlined the general symptoms and indicated the importance of the problem. Fulton and Cromwell (5), in an abstract, briefly described a "black rootrot" of apple found in Pennsylvania and North Carolina which appears very similar to and is probably identical with the disease under discussion. In a preliminary note the writers (3, 4) have stated that the rootrot of apple in Virginia is probably due to one or more species of Xylaria and have designated it "Xylaria rootrot." As a descriptive common name "black rootrot" is preferable and will be used in the belief that the assumed identity of the Virginia and North Carolina rootrot diseases will be confirmed.

The disease is most prevalent in the middle and north "Valley," "Piedmont," and "Appalachian" sections of Virginia, and is probably present to some extent throughout the State. Our information is based on conditions as found in Alleghany, Rockbridge, Albemarle, Nelson, Augusta, Rockingham, Botetourt, Montgomery, and Frederick Counties. Losses of trees ranging between 5 and 25 per cent of the entire orchard have been observed. These losses, together with the high death rate of replants, which will invariably exceed that of the original trees in a stated period of years, make the disease a most formidable one. Financial losses from rootrot are, of course, very considerable.

SYMPTOMS OF ROOTROT

Many of the superficial symptoms of the disease may equally well characterize injuries due to other agencies such as winter injury, malnutrition, attacks of mice or rabbits, tree borers, woolly aphis, crown-gall, and collar-blight; but the combination of all symptoms, particu-

[1] Paper No. 47 from the Laboratories of Plant Pathology and Bacteriology, Virginia Agricultural Experiment Station.

[2] Now Assistant Plant Pathologist, Porto Rico Agricultural Experiment Station.

Journal of Agricultural Research, Washington, D. C.
iv

Vol. X, No. 4
July 23, 1917
Key No. Va. (Blacksburg)—1

larly those of the root system, affords a reliable basis for diagnosis. The first indications of an attack of rootrot are seen in the foliage. The leaves are paler in color than the normal green and have a yellow cast, an appearance of unthriftiness, and are smaller than normal. The thinness of the foliage later attracts attention (Pl. 15, *A*, *B*). This appearance is produced by a failure of many of the lateral buds to develop and by a check in length of the terminal growths. In later stages one or more of the main branches may die, while others may appear normal, but more commonly the general death of the tree results.

Trees of bearing age in late stages of rootrot have a tendency to heavy bearing, the fruit being small and poor in quality. One of the most reliable signs is an inclination of the trunk, the lean being directed away from the most seriously affected roots. Affected trees appear to die rather suddenly, but in most cases symptoms of rootrot have been present for a year or more; trees are rarely, if ever, killed within a single season from the time of infection. This is shown by a marked check in the terminal growth of branches which usually extends back over a period of two or more years and indicates the cumulative effect of a comparatively slow-acting parasite. Measurements of the length of terminal growths of two contiguous 6-year-old York Imperial apple trees, one healthy and the other affected with rootrot, gave the averages shown in Table I. The affected tree shows a marked check in growth beginning in 1915. Plate 15, *E*, shows representative terminal growths of these trees; the check in growth, the suppression of lateral buds, and the decrease in number and size of the leaves are apparent.

TABLE I.—*Average terminal growth of healthy and of diseased York Imperial apple trees*

Year.	Healthy tree.	Rootrot tree.
	Inches.	*Inches.*
1913	11. 25	13. 00
1914	5. 25	7. 83
1915	8. 90	. 75
1916	10. 00	1. 16

Death from rootrot may result at any stage in seasonal development, but the majority of trees probably succumb during the fall or winter. When death takes place during the growing season, the leaves brown and shrivel and hang on for sometime. Trees of any age may be subject to attack. The greater percentage dies between the ages of 12 and 20 years; a few may die as early as five years or younger. Trees on newly cleared land from which the stumps of forest trees have been removed shortly before planting usually die more rapidly and in greater numbers than those planted on cultivated land. A high percentage of the replants set where trees affected by rootrot have been removed die from the same disease (Pl. 17, *B*). Replant death commonly begins at three years of

age, and the percentage of death at seven years may equal or exceed that of the original trees at 15 years. All varieties of the apple probably suffer equally from the disease. We have found it equally prevalent on York Imperial, Winesap, Ben Davis, Black Twig, Grimes Golden, Albemarle Pippin (Yellow Newtown), and other varieties under equal conditions of exposure.

The appearance of roots of affected trees is quite characteristic. Young trees in advanced stages of infection may readily be uprooted by hand. In uprooting, the larger roots break off near the trunk, and often only a few small superficial roots support the tree. The bark, when not too far disintegrated, is covered with a thin black encrustation which is easily peeled off. The wood is brown and is marked with dark-brown zonations. On recently killed roots the wood is firm, and the zonations may penetrate it to some depth. The margin between sound and diseased bark and wood is sharply marked by a change in color, and thin brown strands may extend into the sound tissue for some distance. Sunken, discolored areas are usually found on the surface of the sound root beyond the main lesion (Pl. 17, *C*). They have the appearance of surface infections and may be superficial, or may penetrate to some depth. Usually but little evident mycelium is present on the surface of roots or within them. In advanced stages of decay roots are punky and brittle and vary in appearance; various fungi may be present and acting on them. Rhizomorphoid strands have been found associated with this rootrot in a few cases only, and then on roots in advanced stages of decay. The crown of the tree almost invariably shows the discoloration of the wood and bark found on the roots. The discoloration may extend up the trunk a foot or more above the surface of the ground and is often restricted to one side. Trees showing recognizable foliar symptoms of the disease are found to have the greater part of their root system affected. The initial infection of the root system may take place at any point, and the infection apparently progresses with equal rapidity toward and away from the crown. Infection may be communicated laterally to healthy roots in contact with diseased roots through uninjured rootlets or through wounds in large roots. Infected roots die back to the crown establishing the infection there, and the sound roots become infected subsequently through their attachment with the crown. Death of the tree probably results within a comparatively short time after the infection becomes established in the crown. Figure 1 shows the condition of the root system of a tree which showed pronounced rootrot symptoms.

FUNGI ASSOCIATED WITH ROOTROT

The absence of fruiting stages of fungi on affected roots has been noteworthy. The early removal of diseased trees as practiced in most orchards partly explains this. Perithecial stromata of *Xylaria polymorpha* (Pers.) Grev. have been found on the roots of several affected apple trees in two orchards at Barber (Pl. 15, *C*; 17, *D*), and numerous

stromata of *X. hypoxylon* (L.) Grev. appeared at the base of a dead apple tree at Blacksburg (Pl. 17, *A*). These had produced but few spores at the time of collection (January), but sporulated abundantly after being placed in moist chamber for a month (Pl. 17, *F*). Carpophores of *Clitocybe monadelpha* (Morgan) Sacc. were found at the base of several trees in one orchard at Middletown, but the relation of this fungus to the rootrot problem has not been determined. Affected roots brought to the laboratory and placed in moist chamber have developed conidial stromata of *X. hypoxylon* in a few cases (Pl. 15, *D*).

Isolation studies from roots in various stages of attack have been carried on during the past two growing seasons (1915 and 1916) in continuation of previous studies by Reed and Crabill. Crabill (1) reported the isolation of *Trichoderma koningi* Oud. in culture 72 times in 116 trials, using apple roots from seven sources, and concluded that this fungus is probably the cause of rootrot. No experimental proof of its pathogenicity was given. Jensen (9), Werkenthin (13), and Waksman (12) have found *T. koningi* commonly present in various types of soil, and the writers have found it common on decaying wood. Although we have also frequently isolated this fungus from diseased apple roots, we are convinced from inoculation studies that it exists in them as a saprophyte in tissue killed by other agencies.

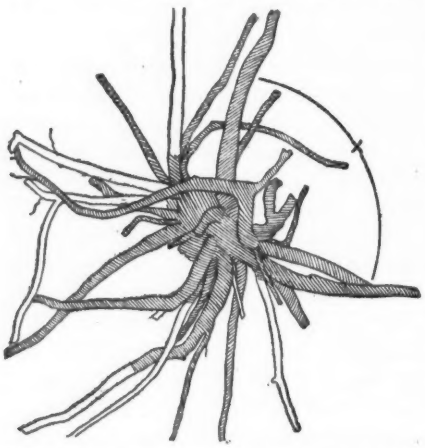


Fig. 1.—Root system of a 7-year-old apple tree affected with rootrot. The infected parts are shaded. Infection was progressing outward on the sound roots. The initial infection probably took place on the side marked *X*. Drawn from a photograph.

In our isolations we have plated fragments of diseased roots on agar media. In the early part of the work the tissue fragments were taken from various parts of roots and a number of species of fungi were obtained, with *Trichoderma koningi* predominating. Later, the fragments were taken only from the margins of the lesions. A considerable number of these have yielded cultures of a species of Xylaria,[1] often uncontaminated with *T. koningi*, the most common accompaniment, and occasional species of Fusarium, unidentified fungi, and bacteria. *Physalospora cydoniae* Arnaud (*Sphaeropsis malorum* Peck) was obtained from the

[1] At first assigned to the genus Stilbella. A culture bearing conidial stromata was submitted to Dr. C. L. Shear, of the Bureau of Plant Industry, who stated that the fungus was probably *Xylaria hypoxylon*.

roots of one tree and from the margin of a trunk lesion extending about 1 foot above the surface of the ground on another. *X. polymorpha* was also obtained from both these sources. An unidentified fungus, probably a basidiomycete, which invests the roots with a conspicuous mat of white mycelium has been cultured from several sources. It has proved slightly, if at all, pathogenic on excised living apple roots in a moist chamber, but its appearance in the field suggests that it may be parasitic in some cases, probably on unthrifty trees.

Cultures of species of Xylaria were obtained during 1915 and 1916 from orchards located at 11 places in Virginia. Three species are represented.

Xylaria hypoxylon has been obtained from nine places: Winchester, Middletown, Pleasant Valley, Staunton, Fishersville, Cloverdale, Blacksburg, Buena Vista, and Greenwood. The isolations from the last two places are tentatively assigned to this species, but differ from the typical *X. hypoxylon* in certain constant cultural features; whether these are of specific or varietal character remains to be determined.

Xylaria polymorpha has been obtained from two orchards at Barber. The identity of the cultures obtained from apple roots has been established by comparison with cultures grown from ascospores taken from mature stromata on apple roots and locust stumps.

Xylaria sp. from Harrisonburg is distinct from the two preceeding species. In culture it resembles *X. cornu-damae* (Schw.) Berk. obtained from germinated ascospores from locust, but its identity with this species is problematical.

CULTURE FEATURES OF THE XYLARIAS

All species of Xylaria grew readily on a variety of nutrient media. Gueguen (6), Harder (8), and Freeman (2) have studied the development of *X. hypoxylon* in culture, and Gueguen (7) has also described pure cultures of *X. polymorpha*. Our experience that stromata of *X. hypoxylon* bearing conidia are produced on a variety of culture media is in agreement with their findings. Freeman, however, states that ascospores were produced in cultures of *X. hypoxylon* on steamed blocks of elm wood in six or eight weeks. We have not succeeded in obtaining ascospores in any of our cultures.

Czapec agar [1] and a starch-agar medium have been used in most of our isolation and culture work, the former being preferable for the production of stromata. The colonies of the three species in petri-dish cultures are easily distinguished. Colonies of *Xylaria hypoxylon* (Pl. 16, *D*) have considerable aerial mycelium with strongly marked, irregularly concentric zones, irregularly lobed margins, and dark pigmentation. Colonies of *X. polymorpha* and *Xylaria* sp. have appressed mycelium with

[1] Cane sugar, 30.0 gm.; magnesium sulphate, 0.5 gm., potasium chlorid, 0.5 gm; potassium acid phosphate, 1.0 gm.; sodium nitrate, 2.0 gm.; iron sulphate, trace; agar, 20.0 gm.; water, 1,000 c. c.

indistinct zonation, entire or slightly lobed margins, and produce pigment only with age or not at all. Colonies of *Xylaria* sp. enlarge much more slowly than those of the two other species. The zonation of colonies of *X. hypoxylon* results from the formation of aerial mycelium at the growing margins followed by appressed mycelium. The pigment, which is at first greenish and becomes almost black with age, forms first in the central zone and appears in the other zones successively from the center outwards. The upright stromata begin to form when the colonies are about 2 weeks old and may appear at the center or on the margins of the zones. They vary in numbers from 2 or 3 to 20 or more. The stromata of *X. hypoxlon* are borne singly or in groups and may become 4 cm. or more in length, with a diameter of 2 or 3 mm. (Pl. 17, *E*). Conidia are produced at the upper extremities in four or five weeks. Cultures of *X. polymorpha* and *Xylaria* sp. have commonly produced only rudimentary stromata. One culture of *X. polymorpha* has, however, developed stromata 5 cm. long and 2 mm. broad. Gueguen's (7) experience that the development of *X. polymorpha* in culture is correlated with its seasonal development in nature is of interest in this connection. A more detailed study of the production of stromata and conidia is reserved for a later publication.

PATHOGENICITY OF THE XYLARIAS

Little is known of the exact mode of life of the Xylarias in nature. It has probably been assumed very generally that they exist as saprophytes; and the appearance of their fructifications on stumps, fence posts, and similar situations is evidence that they are commonly saprophytic. We have found but one reference which bears on the question of parasitism. Harder (8) obtained evidence of the pathogenicity of *X. hypoxylon* when inoculated into small wounds in short pieces of living 1-year-old shoots of two trees (the species were not named). After three months in moist chambers the shoots were still living, as shown by the development of buds and roots, the bark at the point of inoculation was browned to a distance of 3.5 cm., and the wood beneath was brown and dead. The inoculum was recovered.

Our inoculations were made on living apple roots and branches in moist chambers and in the field, also on roots of peach (*Amygdalus persica*), hawthorn (*Crataegus spathulata*), red oak (*Quercus rubra*), white oak (*Q. alba*), and walnut (*Juglans nigra*) in moist chambers.

MOIST-CHAMBER INOCULATIONS

Pieces of roots or branches about 6 inches long and 1 inch in diameter were used. After a preliminary washing and sterilization in alcohol a small notch was cut with a sterile knife and the inoculum of mycelium from the growing margin of a colony introduced. Moisture was main-

tained with a layer of sterilized sand. Roots of apple, peach, hawthorn, and walnut remained alive and in good condition in these moist chambers for more than three months, often sending out shoots several inches long. The oak roots did not keep so well and showed no adventitious budding. One series included inoculations with isolations of *Xylaria hypoxylon* from seven sources and with *Xylaria* sp. Each moist chamber contained one inoculated apple root, one control root wounded but not inoculated, and one inoculated apple limb. All the strains of *X. hypoxylon* proved pathogenic on apple roots and limbs. On roots the progress of infection was seen in the production of surface mycelium on the bark (Pl. 16, *F*) radiating outward from the point of inoculation accompanied by drops of brown exudate and some splitting of the bark. The mycelium, at first white, later developed pigment and formed a black stromatic crust over the surface of the root, typical of that found on affected apple roots in nature. The surface mycelium and crust developed most vigorously in a saturated atmosphere. After six weeks the pieces were removed and split longitudinally through the wound. The bark and wood were brown and dead to some distance from the wound (Pl. 16, *A*). The smallest lesion produced extended 1 cm. and the largest 9 cm. proximally and distally from the point of inoculation. The depth of the lesions varied from 3 mm. to 1.5 cm., sometimes extending to the center of the root. Abundant mycelium was found in the vascular bundles, while but little was present in the medullary rays. All the control roots remained healthy. Additional inoculations have confirmed these results. The inoculum has been recovered with ease whenever attempted.

On limbs the mycelium of *Xylaria hypoxylon* produced more conspicuous surface mats than on the roots (Pl. 16, *B*) and from which the black encrustation developed (Pl. 16, *C*). Six-inch lengths of limbs were completely covered with the mycelium in less than six weeks; the wood, however, was only penetrated to a depth of a few millimeters. *Xylaria* sp. proved almost as vigorous a parasite as *X. hypoxylon*. In 10 weeks the root infection had spread 2 cm. proximally and distally and 5 mm. inward, while that on the limb had advanced 3 cm. in the bark and had penetrated the wood 2 mm.

Xylaria hypoxylon proved only slightly pathogenic on roots of red oak, white oak, and walnut. A heavy black crust was formed over the wound, but the wood was penetrated but slightly after three months. The hawthorn root was attacked somewhat more vigorously, the wood having been penetrated to a distance of 1 cm. in three months.

Mycelium of *Xylaria polymorpha* produced only slight penetration of the bark of apple roots after three months, and similar results were obtained with peach roots. Ascospores sown on the wound surface of an apple root produced a small amount of mycelium which did not penetrate the sound wood.

Trichoderma koningi, when placed on wounded living apple roots in a moist chamber, grew for a time on the medium transferred, but produced no penetration of the root. *Sphaeropsis malorum*, however, proved pathogenic on apple roots, but the infection progressed more slowly than that produced with *Xylaria hypoxylon*. The lesion had extended 1.5 cm. proximally and distally and to a depth of 5 mm. after four weeks (Pl. 15, *E*). Recovery in pure culture was obtained.

FIELD INOCULATIONS

Results similar to those obtained in moist chambers have been obtained in inoculations of living apple roots in the field. A number of 1-year-old apple trees were inoculated with *Xylaria hypoxylon* through a small bark wound near the base of the main root and then planted. A few of these examined after four months all showed infection. One bore a lesion 1.5 cm. long which had penetrated to the center of the root. *X. hypoxylon* was recovered from the margin of the lesion. A number of roots of a 20-year-old apple tree were cut across about 5 feet from the trunk, and mycelium of *X. hypoxylon* was inserted beneath the bark at the cut end. The wound was covered with cotton and the soil replaced. Two of these roots examined after seven weeks had been killed 4 cm. from the wound and bore the typical black encrustations on the surface. Another series of roots were inoculated through small bark wounds. After five weeks infection was well established and was advancing into living tissue.

CONCLUSIONS FROM INOCULATION STUDIES

Xylaria hypoxylon has proved to be an active wound parasite on apple roots; *Xylaria* sp. from Harrisonburg is also a wound parasite, and *X. polymorpha* appears to be only slightly or occasionally parasitic. *Sphaeropsis malorum* is the only other fungus obtained from apple roots that has proven parasitic. The isolation of *X. hypoxylon* from roots of affected trees from 9 of the 11 orchards under observation and the experimental evidence of its pathogenicity, accompanied by the production of symptoms in inoculated roots typical of those found in the orchard, indicate that this fungus is the common cause of the black rootrot of the apple in Virginia. Confirmatory evidence must be obtained in the production of typical symptoms and death in growing apple trees from pure-culture inoculations. This is provided for in a series of 500 young apple trees inoculated and planted during the past year (1916) at Blacksburg. Positive results from these inoculations can not be expected for a year or more, since the disease progresses slowly in nature.

CONTROL OF BLACK ROOTROT

No control measures of proved value are known. There is little possibility of effecting the cure of diseased trees. Those showing but

slight foliage symptoms of rootrot are found to have the infection of the root system so well established that the removal of infected parts from the tree and soil seems a practical impossibility. Control measures must therefore be directed toward the prevention of replant infection and further spread of the infection to healthy trees. Experiments with soil disinfection on a small scale offered but little promise. Replants set after soil treatment with some of the common disinfectants died as rapidly as those on untreated soil. Enough work on this line has not been carried on, however, to warrant general conclusions. Tests of the susceptibility of different rootstocks is suggested as a promising line of experiment.

It has been the practice among some orchardists to lime the soil from which trees affected with rootrot were removed before replanting. We have not secured sufficient data to judge of the value of this treatment, but it seems probable from laboratory studies that an alkaline soil is less favorable for the growth of species of Xylaria than an acid soil. The radius of colonies of *X. hypoxylon* after three weeks' growth on starch-agar media, which ranged in reaction between $-20°$ and $+10°$ Fuller's scale was as follows: $-20°=2.6$ cm.; $-10°=3.0$ cm.; neutral$=$ 3.0 cm.; $+10°=4.5$ cm. Similarly the addition of hydrated lime ($Ca(OH)_2$) to the medium produced a check in the growth of colonies of *X. hypoxylon*, the radius of the colony on the untreated medium at 17 days being 4.0 cm., while that on the medium receiving hydrated lime was 2.5 cm.

Field observations indicate that infection is commonly distributed through the orchard in cultivation. The establishment of quarantined areas, where the disease is confined to limited blocks of trees, and the withholding of cultivation within these areas, thus preventing the carrying over of infective material to the noninfected areas, should prove of value in some cases. The removal of borers without disinfection of the knife may readily transmit infection from diseased to healthy trees. It further seems quite probable that the infection of healthy trees may result from the intermingling of their roots with those of diseased trees, but infection would probably progress slowly in this manner.

The Xylarias are commonly present on stumps of forest trees, and rootrot is consequently most severe on newly cleared land. It seems that the use of cultivated land only for orchard sites may be of considerable practical importance as a preventive measure.

SUMMARY

Black rootrot of the apple is an infectious disease of very considerable economic importance which has become a serious menace in the chief orchard sections of Virginia.

Foliage symptoms of the disease are not markedly different from those produced by injuries due to other agencies, but the black encrustations

on the surface of affected roots and the accompanying dark zonations in the bark and wood are reliable diagnostic features.

Field observations show that rootrot is infectious. The progress of the disease indicates an attack of a comparatively slow working parasite. Two or more years of infection are probably required to produce the death of the tree.

Apple trees planted on newly cleared land are more liable to attack than those on land cleared and cultivated for some time before planting.

Three species of Xylaria have been obtained in pure culture from affected apple roots. *X. hypoxylon* obtained from orchards at nine places proved to be an active wound parasite on living apple roots in moist chambers and in the field. Typical black rootrot symptoms were produced and recovery in pure culture was obtained. *Xylaria* sp. (undetermined) from one source also proved pathogenic. *X. polymorpha* from two orchards at one place proved only slightly, if at all, pathogenic. *X. hypoxylon* is probably the chief cause of black rootrot of the apple in Virginia.

Exclusion practices are suggested as control measures of probable value.

LITERATURE CITED

(1) CRABILL, C. H.
1916. NOTE ON APPLE-ROT IN VIRGINIA. *In* Phytopathology, v. 6, no. 2, p. 159–161, 1 fig.

(2) FREEMAN, Linford.
1910. UNTERSUCHUNGEN ÜBER DIE STROMABILDUNG DER XYLARIA HYPOXYLON IN KÜNSTLICHEN KULTUREN. *In* Ann. Mycol., v. 8, no. 2, p. 192–211, 14 fig., pl. 4 (col.).

(3) FROMME, F. D., and THOMAS, H. E.
1917. THE ROOT-ROT DISEASE OF THE APPLE IN VIRGINIA. *In* Science, n. s., v. 45, no. 1152, p. 93.

(4) ——— ———
1917. A XYLARIA ROOT-ROT OF THE APPLE. (Abstract.) *In* Phytopathology, v. 7, no. 1, p. 77.

(5) FULTON, H. R., and CROMWELL, R. O.
1916. BLACK ROOT-ROT OF THE APPLE. (Abstract.) *In* Phytopathology, v. 6, no. 1, p. 110.

(6) GUÉGUEN, Fernand.
1907. RECHERCHES BIOLOGIQUES ET ANATOMIQUES SUR LE XYLARIA HYPOXYLON. *In* Bul. Soc. Mycol. France, t. 23, fasc. 4, p. 186–217, pl. 21–22.

(7) ———
1909. L'ÉTAT CONIDIEN DU XYLARIA POLYMORPHA GREV. ÉTUDIÉ DANS SES CULTURES. *In* Bul. Soc. Mycol. France, t. 25, fasc. 2, p. 89–97, pl. 4.

(8) HARDER, Richard.
1909. BEITRÄGE ZUR KENNTNIS VON XYLARIA HYPOXYLON (LIN.). *In* Naturw. Ztschr. Forst. u. Landw., Jahrg. 7, Heft 8, p. 429–436, fig. 1; Heft 9, p. 441–467, fig. 2–17.

(9) JENSEN, C. N.
1912. FUNGUS FLORA OF THE SOIL. New York (Cornell) Agr. Exp. Sta. Bul. 315, p. 413–501, fig. 100–134.

(10) REED, H. S., and CRABILL, C. H.
1913. PLANT DISEASES IN VIRGINIA IN THE YEARS 1911 AND 1912. *In* Va. Agr. Exp. Sta. Rpt. 1911/12, p. 35–50, fig. 2–14.

(11) ——— ———
1915. NOTES ON PLANT DISEASES IN VIRGINIA OBSERVED IN 1913 AND 1914. *In* Va. Agr. Exp. Sta. Rpt. 1913/14, p. 37–58, 17 fig. (Tech. Bul. 2.)

(12) WAKSMAN, S. A.
1916. SOIL FUNGI AND THEIR ACTIVITIES. *In* Soil Science, v. 2, no. 2, p. 103–155, 5 pl. Literature cited, p. 148–155.

(13) WERKENTHIN, F. C.
1916. FUNGUS FLORA OF TEXAS SOILS. *In* Phytopathology, v. 6, no. 3, p. 241–253.

PLATE 15

A.—A healthy 6-year-old apple tree contiguous to the affected tree shown in figure *B*, which appears in the background.

B.—A 6-year-old apple tree showing pronounced symptoms of rootrot in the thinness of the foliage and inclination of the trunk.

C.—*Xylaria polymorpha* fruiting on large lateral root of a 7-year-old apple tree.

D.—*Xylaria hypoxylon* developing stromata on an apple root after three months in a moist chamber.

E.—Terminal growths of trees shown in figures A and B, showing effect of rootrot on annual increase in length: *a*, branch from the tree affected by rootrot; *b*, branch from the healthy tree.

A
B
C
D
E
1916
1915
1914
1913
1916
1915
1914
1913
a
b

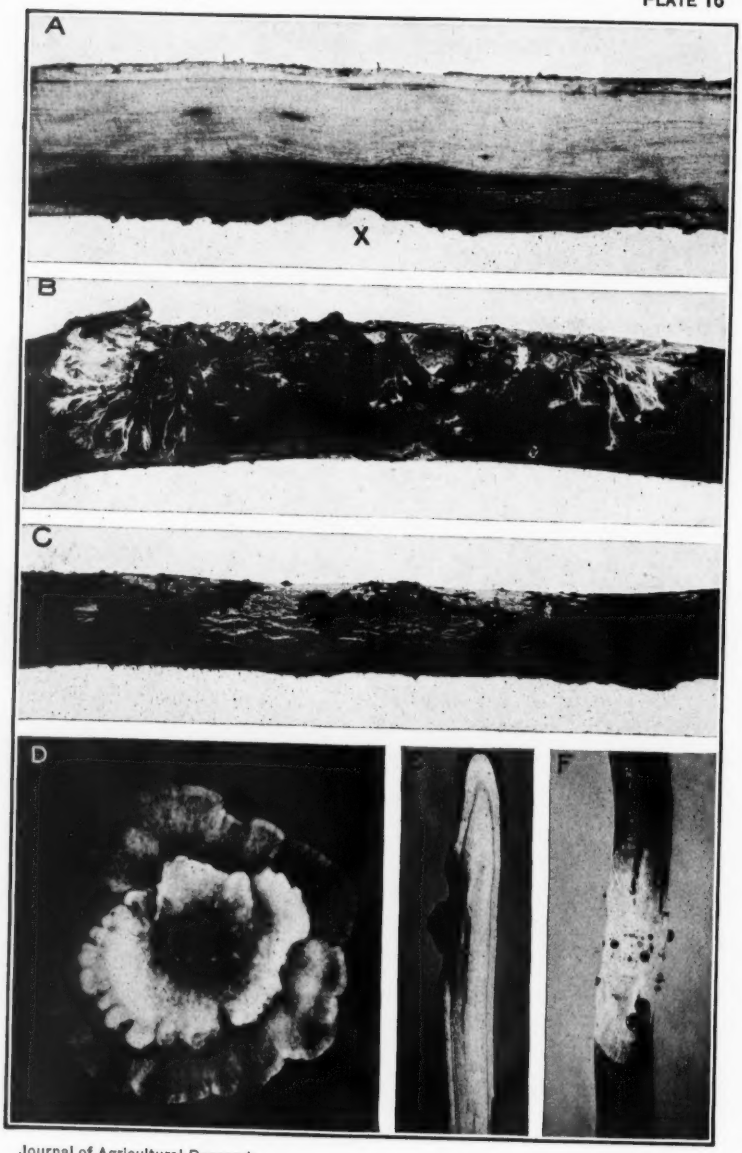

A
X
B
C
D
F

PLATE 16

A.—A longitudinal section of a living apple root inoculated at *X* with mycelium of *X. hypoxylon* showing discoloration of wood and bark, after six weeks in a moist chamber.

B.—Surface view of a living apple limb inoculated with *X. hypoxylon* after five weeks in moist chamber.

C.—Black encrustation produced on an apple limb inoculated with *X. hypoxylon* in a moist chamber.

D.—An 8-day old colony of *X. hypoxylon* on starch agar showing characteristic zonation and lobed margin.

E.—A longitudinal section of a living apple root inoculated with *Sphaeropsis malorum*, after four weeks in a moist chamber. A black mat of mycelium has formed over point of inoculation.

F.—A surface view of a living apple root inoculated with mycelium of *X. hypoxylon*, after four weeks in moist chamber.

PLATE 17

A.—*Xylaria hypoxylon* fruiting at the base of a dead apple tree.

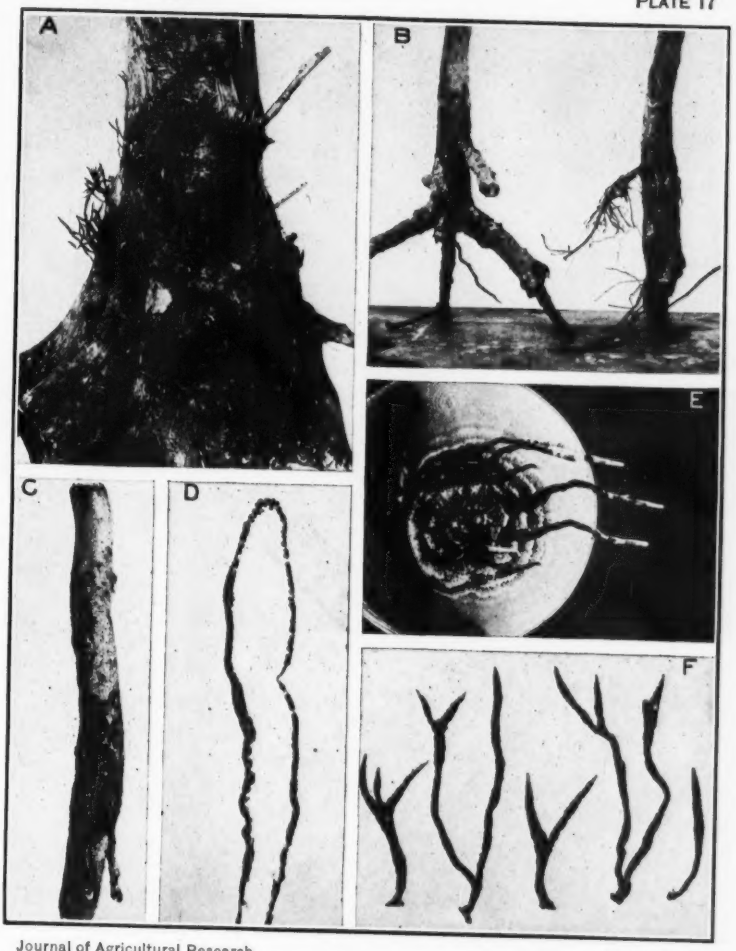
B.—Stumps of young apple trees which have died from rootrot after having been planted where old trees had died from the same cause. Note black encrustations. Photographed by Dr. H. S. Reed.

C.—Black rootrot lesions on an apple root from Cloverdale, Va. The margin between diseased and sound parts is sharply marked. *X. hypoxylon* was obtained from these lesions.

D.—Longitudinal section of a stroma of *X. polymorpha*, showing perithecia embedded in the periphery.

E.—Conidial stromata of *X. hypoxylon* in petri-dish culture on Czapec agar. The direction of growth is positively phototropic.

F.—Mature stromata of *X. hypoxylon* from stump shown in figure A, producing ascospores after one month in a moist chamber.


A
B
C
D
E
F

PHYSIOLOGICAL EFFECT ON GROWTH AND REPRODUCTION OF RATIONS BALANCED FROM RESTRICTED SOURCES

By E. B. HART, E. V. MCCOLLUM, and H. STEENBOCK, *of the Department of Agricultural Chemistry*, and G. C. HUMPHREY, *of the Department of Animal Husbandry, Wisconsin Agricultural Experiment Station*

Our early work (6)[1] on the nutrition of Herbivora with restricted rations demonstrated clearly the inadequacy of the accepted theory as to what constitutes a balanced or complete ration. Up to that time total protein (without reference to quality), energy, and ash materials were considered the essentials of a ration. The latter, however, occupied no position in the mathematical expression of the standards developed. The standards have been stated only in terms of total digestible protein and energy. It is, however, probably true that in a practical sense and with the generally accepted knowledge of the quality of feeding materials accumulated from a long and varied experience, such standards have had and will continue to have very great value; but their limitations are also made evident by this earlier work and are emphasized by what we have since done. Within the past few years our knowledge (1, 2, 7, 9, 10, 13, 16, 17) of the essentials of a ration have expanded, and to-day we would consider a ration complete and efficient only when it contained protein of adequate quantity and quality, adequate energy, ash materials in proper quantity and proportion, and two factors of unknown constitution (vitamines), designated by this laboratory (11) "fat-soluble A" and "water-soluble B."

In addition to the above normal factors, there may be introduced with natural foodstuffs the important factor of toxicity (4, 5, 12). This can be wholly absent or so mild in its effects as to be entirely obscured when the other essentials of a ration are at an optimum adjustment; or with fair adjustment it may only reveal its effects when the ration is continued over a very long time and the animal involved in the extra strains of reproduction and milk secretion. This resistance to toxicity is very materially increased through a proper adjustment of the normal factors of nutrition.

With this recognition of all the normal factors for adequate nutrition there should not arise simultaneously a desire for a mathematical expression of these factors in feeding standards. It is doubtful if this can ever be done, at least for certain of them. For example, the rôle of the mineral nutrients is so varied, including such widely separated functions

[1] Reference is made by number to "Literature cited," p. 197–198.

as construction and control through antagonism as to make it seem futile to attempt an expression of absolute requirements when natural foods, with their diversity of mineral content, are involved. Even the recognition of differences in the quality of proteins and their relation to nutrition (3, 8, 14) will make it more difficult to continue expressing protein requirements in exact quantities than before the development of such knowledge; and what can be said of the quantitative requirements of fat-soluble A and water-soluble B and their supply in feeding materials?

All these developments of the last few years emphasize the need of a thorough study of the contributing nutritive factors of a single foodstuff, and in the state of our present knowledge such information will be secured only by physiological tests involving the animal in reproduction and milk secretion. A contributing factor by a natural food may at times be in the nature of toxicity and this may serve as a harmful and abnormal factor. As such knowledge develops and it becomes clear that this or that single food material will supply adequately the normal nutritive factors not measurable by any quantitative chemical method, such as fat-soluble A, water-soluble B, or mineral nutrients, then we will return with more confidence to the mathematical standard that involves only the energy and protein supply of that single food material. This confidence in the expressed quantities of energy and protein available in a foodstuff will rest upon the definite information that they become physiologically effective only when they form part of a ration which carries one or a number of foodstuffs supplying adequately the other nutritive factors. With such an understanding the feeding standards developed on the energy-protein basis would continue to be theoretically sound and of very great practical value. As illustrative of our position, and taken from our own experience with wheat-grain feeding, we would feel reasonably safe if a wheat-grain ration, based on protein and energy and to be fed continuously to a growing herbivorous animal, was built around alfalfa hay, less safe if built around corn stover, and fearful of disaster should the roughage used be wheat straw. These facts should emphasize the very great necessity for the accumulation of information on the nutritive value of single foodstuffs, and it is apparent that such information will become very valuable in the future use of the protein-energy standards. Such knowledge will not destroy, but will supplement the feeding standards of the present time and secure more confidence in their use.

EXPERIMENTAL WORK

GROWTH ON WHEAT AND CORN RATIONS

For purposes of locating the deficiencies of the all-wheat-plant ration (wheat grain, wheat gluten, and wheat straw), which had given fair growth, but was a failure in reproduction with grade Shorthorn heifers,

a series of experiments was again started in 1910, using for the purpose vigorous grade Holstein heifers of initial weights of from 200 to 400 pounds. It was also proposed that one group should receive its nutrients wholly from the corn plant, another from the wheat plant, a third from corn grain and wheat straw, a fourth from wheat grain and corn stover, and a fifth group should receive its nutrients from corn grain with the roughage equally divided between alfalfa hay and wheat straw. These rations were closely comparable in digestible proteins and net available energy and were "balanced" in the ordinary sense of the standards. The animals were fed what they would consume of this mixture and in addition received common salt and natural water. They were allowed a daily run to an outside paddock free from all vegetation.[1] Their records of growth and final status are given in Table I.

TABLE I.—*Record of growth of Holstein calves, 1910–1912*

No. of animal.	Ration.	Weight (in pounds).					Condition after 2 years.
		Initial (June 2, 1910).	After 6 months on ration.	After 1 year on ration.	After 18 months on ration.	After 2 years on ration.	
629	Ground wheat, 8 pounds Wheat gluten, 0.3 pounds Wheat straw, 5.7 pounds	377	655	569	610	452	Miserably emaciated.
639	do	406	722	683	630	519	Do.
637	Wheat grain, 6.7 pounds Wheat gluten, 0.3 pounds Corn stover 7 pounds	206	369	533	656	790	Fairly strong.
641	do	207	377	594	783	820	Do.
575	Corn meal, 5 pounds Gluten feed, 2 pounds Corn stover, 7 pounds	349	664	970	1,139	974	Strong and vigorous.
594	do	270	496	735	905	923	Do.
635	Corn meal, 5 pounds Gluten feed, 3 pounds Wheat straw, 6 pounds	208	301	480	591	690	Poor growth and poor condition.
636	do	220	384	541	684	642	Do.
642	Corn meal, 5 pounds, Gluten feed, 2 pounds Wheat straw, 3.5 pounds Alfalfa hay, 3.5 pounds	.....	[a] 613	686	911	1,161	Strong and vigorous.
643	do	.....	[a] 537	602	800	768	Do.

[a] Initial weight.

This breed was more sensitive to the all-wheat ration than our earlier records (6) showed for the Shorthorn cattle, or at least these individuals were. Sustained growth was not possible on this ration. After reaching weights of 600 or 700 pounds both individuals (No. 629 and 639) began to decline in weight and passed into a miserable condition (Pl. 18); they failed to show œstrus and consequently could not be bred. This same group (wheat grain plus wheat straw), if slightly excited or hurried would collapse and remain in a prostrated position for a few minutes, suffering muscular rigor and tremor. From this condition the animals would gradually recover, appearing normal only after a lapse of 10 or

[1] Very great credit is due Mr. William Voss for his constant and intelligent care of these animals.

15 minutes. One of them became blind. The critical factors in this ration were poor mineral content and toxicity. This statement is based on the records made by other animals of this species (to be described later in this paper) and on records with rats and swine (5, 12).

Plates 18 to 22 illustrate the condition of all lots as calves and at the end of two years' feeding.

In contrast to the all-wheat-ration group stood the all-corn-ration group. The latter not only showed continuous growth, but became physiologically active and produced strong calves. The decline in weight at the end of two years shown by No. 575 was due to slow recovery after calving.

When the ration consisted of wheat grain and corn stover, fair but sustained growth was obtained; but it was below normal. The marked improvement made by the use of another roughage, contributing at least as one factor a better mineral content, was most illuminating. These animals showed œstrus and were bred.

When the ration was made from corn grain and wheat straw, slow growth continued for a long time, but the animals were in poor condition. They were better than the wheat-grain and wheat-straw group, since we were able to get one bred, although the other failed to show œstrus. As will be more fully demonstrated later, the critical factor was the mineral content of this ration, and these results serve to emphasize how important a factor the mineral side of a ration becomes in reproduction.

Strong and vigorous individuals resulted where a corn-grain plus wheat-straw plus alfalfa mixture was used. The displacement of half of the wheat straw with alfalfa hay changed the ration from a failure to a success. It might be assumed that the introduction of alfalfa hay corrected the ration by the addition of more or different proteins, but it is more probable that its chief but not only contributing factor in this case was a more efficient ash mixture.

In Table II are recorded the reproduction records of these animals, as well as those of five older cows, reserved from our older herd, the records of which were made public by the writers (6) in 1911. The constituents of the ration fed the older cows were in the same proportions as used for the younger heifers, as shown in Table I. For breeding these animals a Guernsey bull was used in the case of all the old individuals, but a Holstein bull served the 10 Holstein heifers. In addition to the records of birth, there is given in the last column the average daily weight of milk produced for 30 days.

TABLE II.—*Records of reproduction and milk secretion*

CALVES

No. of cow.	Ration.	Sex.	Weight at birth.	Number of days before time of calving.	Condition of calf.	Milk record, daily average for 30 days.
			Pounds.			*Pounds.*
629	All wheat				Cows did not breed.	
639	do					
637	Wheat grain plus corn stover.	Bull	82	15	Weak, grew strong; peculiar deflection of head.	15.9
641	do	Heifer	65	8	Strong	18.9
575	All corn	Bull	85	8	do	24.8
594	do	Heifer	95	6	do	22.7
635	Corn grain plus wheat straw.				Cow did not breed.	
636	do	Bull	70	18	Dead at birth.	11.9
642	Corn grain plus wheat straw plus alfalfa.	Heifer	88	6	Strong	26.0
643	do	do	71	13	Fairly strong	27.2

OLDER COWS

562	Corn grain plus wheat straw.	Bull	47	27	Weak, died	18.4
567	do	do	54	23	do	19.9
570	do	do	68	10	do	15.6
563	All corn	Heifer	65	5	Strong	22.6
572	do	Bull	84	0	do	24.4

The splitting of the ration disclosed two things of importance for our understanding. First, that disaster would follow the use of a corn-grain and wheat-straw ration, indicating that the failure of a wheat-grain and wheat-straw ration was partially to be attributed to the straw. The second fact of importance was that a wheat-grain and corn-stover ration was not perfectly complete, giving at times weak offspring and also indicating that the wheat grain was a contributor to the failure of a wheat-grain and wheat-straw ration. In addition to Table II, there are added a number of illustrations (Pl. 23, 24, 25, *A–B*) showing the condition of the mothers and the calves at date of birth.

Attention should be called to the peculiar limpness and weakness of calves born on a wheat-grain and corn-stover ration. Occasionally such individuals would be able to get up after birth, nurse, and take care of themselves; but more often they would lie stretched out limp and unable to hold their heads in an upright position and would have died had they not been fed by an attendant. Lying in this prostrated position, the head was often thrown back until it rested against the

shoulder, or when moved, wabbled to the other side, making deflections and contortions not unlike those made by pigeons suffering from polyneuritis. Fed the mother's milk with the continuance of the same ration this condition usually disappeared after two weeks, and the calf continued to grow normally. On this ration (wheat grain plus corn stover) the mothers appeared perfectly normal, although we have no evidence that they were absolutely so. The depressing factor of the ration (probable toxicity of the wheat grain) was having its greatest effect on the offspring and presumably through the placenta. It is an interesting fact that this pathological condition could apparently be cured with the milk of the same mother on the same ration. By the use of the milk from the mother the weakness would gradually disappear, and in two to three weeks some calves if born alive from a cow fed a wheat-grain and corn-stover ration would appear normal, while invariably a calf fed a corn-grain and wheat-straw ration could not be saved. We would have to assume as a logical explanation of the revival of the offspring with the mother's milk that during intrauterine life the placental membrane was traversed by the toxic factor, while the mammary cells were not, or that the milk furnished a more perfect nutritive medium than the blood stream, thereby providing for more effective resistance to the action of the toxic material than a poorer ration could. The latter hypothesis is in our judgment less tenable.

INFLUENCE ON MILK SECRETION AND REPRODUCTION OF SALTS ADDED TO IMPERFECT RATIONS

From analytical data we early recognized that a wheat and wheat-straw ration differed materially in its mineral content from a corn and corn-stover ration (6, p. 138). The bases of these diets (calcium, magnesium, and potassium) were considerably lower in quantity in the wheat ration than in the corn ration. In fact, animals on a wheat ration always produced a urine acid to litmus, while on a corn ration the reaction with the same indicator was alkaline. In 1911 we presented a limited amount of data upon the effect of adding calcium, magnesium, and potassium carbonates to a wheat and wheat-straw ration in such quantities as to make the intake of bases practically identical with their quantity in a corn ration. We have again done this with a number of animals, and in addition have supplied the bases as salts of organic acids. It was believed that there might be some additional disturbance created by the continued use of alkaline carbonates and their tendency to keep the contents of the first portion of the digestive tract in alkaline condition, which might explain our failure to correct the disastrous results with the wheat and wheat-straw ration. Consequently quantities of bases equivalent to those added as carbonates were added as calcium lactate, magnesium citrate, and potassium citrate. In some rations only a single salt, such as calcium lactate or potassium citrate,

was added, in which case the amount was made equivalent in basicity to the basicity of a mixture of the three salts. While, of course, single salt additions did not make the ash of the wheat and wheat-straw ration comparable with that of the corn ration, it did serve to correct its acidity.

We have also stated that failure in reproduction resulted when a corn-grain and wheat-straw ration was used. If our theory was correct, that the main deficiency of this ration was a proper mineral content, then salt additions could be made with normal results; and such was actually the case. In Table III are brought together the records of such studies. The amounts of carbonates added to 14 pounds of the ration were 13 gm. of calcium carbonate, 21 gm. of magnesium carbonate, and 47 gm. of potassium carbonate. In the case of the organic salts we used 40 gm. of calcium lactate [$Ca(C_3H_5O_3)_2+5H_2O$]; 60 gms. of magnesium citrate [$Mg_3(C_6H_5O_7)_2+14H_2O$]; and 80 gm. of potassium citrate ($K_3C_6H_5O_7+H_2O$). When calcium lactate was used alone 232 gm. were added to 14 pounds of the ration; where the salt was magnesium citrate alone 181 gm. were used, and where potassium citrate was the only salt 163 gm. were employed. In Table III the numbers of the cows may appear repeated, owing to the fact that it records several gestation periods of a single individual. In addition to the table, Plate 25, *C–G*, is added to illustrate the condition of both mothers and calves on some of the rations.

TABLE III.—*Records of reproduction and milk secretion*

No. of cow.	Ration.	Sex of calf.	Weight at birth.	Number of days before time of calving.	Condition of calves.	Milk record, average daily yield for 30 days.
			Pounds.			*Pounds.*
645	Wheat plus wheat straw plus inorganic mixture.	Heifer...	74	4	Dead at birth.....	15.9
644	.....do.....	.....	.....	.....	Cow would not breed.	.....
567	Wheat, wheat straw, organic salts.	Bull.....	40	98	Dead at birth.....	(a)
570	.....do.....	Heifer...	72	15	Weak, died at 72 hours.	18.9
646	Corn grain plus wheat straw plus organic salts.	...do.....	75	16	Strong; lived.....	26.21
647	.....do.....	...do.....	72	22	.....do.....	28.2
636	.....do.....	...do.....	83	16	.....do.....	26.6
570	Wheat grain plus wheat straw plus calcium lactate.	.....	.....	.....	Cow would not breed.	.....
562	.....do.....	.....	.....	.....	.....do.....	.....
637	Wheat plus wheat straw plus magnesium citrate.	Bull.....	33	69	Dead at birth.....	(a)
641	.....do.....	...do.....	37	65	.....do.....	(a)

(a) No milk.

TABLE III.—*Records of reproduction and milk secretion*—Continued.

No. of cow.	Ration.	Sex of calf.	Weight at birth.	Number of days before time of calving.	Condition of calves.	Milk record, average daily yield for 30 days.
			Pounds.			*Pounds.*
645	Wheat grain plus wheat straw plus potassium citrate.	.....	.....	.....	Cow would not breed.	.....
644	.....do.....	.....	.....	.....	.....do.....	.....
636	Wheat grain plus wheat straw plus organic salts.	Heifer...	59	38	Dead at birth.....	(a)
651	.....do.....	Bull.....	54	35	Weak; died.....	11.9
637	Wheat grain plus corn stover.	...do.....	66	26	.....do.....	26
641	.....do.....	...do.....	82	18	Strong; lived.....	25.3

a No milk.

Table III illustrates certain principles very clearly. It shows that it was impossible to make the wheat-grain and wheat-straw ration complete by additions of salts whether they were inorganic or organic in character. Single additions also were a failure and even disturbed in some instances the breeding potency. These records make it clear that an acid condition was not the only disturbing factor of an all-wheat ration, but that there was some other factor at work, resident in the grain. The substitution of the corn grain for the wheat grain made the ration physiologically perfect where, in addition to the straw, certain salt additions were made. The calves produced by the mothers on this latter ration were normal and the milk secretion good. The history of No. 636 is particularly instructive in this connection. As seen in Table II, on a corn-grain and wheat-straw ration her calf was dead and she secreted but 11.9 pounds of milk daily. On a corn-grain plus wheat-straw plus organic-salt mixture (Table III) the calf was born strong, lived, grew up, and was finally sold. On this ration the average daily milk secretion reached 26.6 pounds. Again, on a wheat-grain plus wheat-straw plus organic-salt mixture (Table III) a dead calf was produced by the same cow and little or no milk secreted.

In addition to the records of the effect of additions of a salt there are shown records of No. 637 and 641, again illustrating the effect of adding corn stover to the wheat grain. In the case of one individual, No. 641, a strong calf resulted, while this same cow on the wheat plus wheat-straw plus magnesium-citrate ration produced a dead calf. In the case of the other cow, No. 637, the corn stover did not completely overcome the toxicity of the wheat grain, although it accomplished much more than mere additions of a salt to a wheat-grain and wheat-straw ration could, as evidenced by the greater length of intrauterine life. Illustrations of cows and calves under the influence of these rations are given in Plate

26, *A–C*. With the addition of the organic salts to the wheat-grain and wheat-straw ration the mature mothers remained in apparently good condition for a very long time, as shown in the illustration of No. 570 (Pl. 25, *E–G*).

These records demonstrate clearly that in the wheat-grain and wheat-straw ration at least two factors were operative against normal nutrition: Poor ash and toxicity; but that a corn and wheat-straw ration could be made complete by a suitable ash adjustment, while a wheat and wheat-straw ration could not.

EFFECT ON REPRODUCTION OF BAKING THE WHEAT

The extraordinary facts developed in these inquiries that the whole-wheat grain, unless accompanied by certain roughages, was harmful to growth and reproduction suggested inquiry into the possible effect of baking on these nocuous properties. The fact that baking is always resorted to in the preparation of either bolted-flour bread or whole-wheat bread made this phase of the problem important from the standpoint of human nutrition.

Consequently the grain mixture of the all-wheat ration (wheat grain plus wheat gluten) was sent to a baker, there mixed with water to a dough, molded into loaves, and baked at the usual baking temperature for ordinary white bread. It was then broken up, reground, and fed with wheat straw alone, with straw and organic salts, or with corn stover. In addition to the data on baked wheat, there are also added two records of a medium-grade bolted flour used with corn stover. This flour was of a grade commonly used by the bakers of Madison for bread making. The records of reproduction on these rations are shown in Table IV. Plates 26, *D–F*, and 27 illustrate the condition of the calves and cows receiving these rations.

TABLE IV.—*Record of reproduction and milk secretion with baked wheat and raw flour*

No. of cow.	Ration.	Sex.	Weight at birth.	Number of days before time of calving.	Condition of calf.	Milk record (average daily yield for 30 days).
			Pounds.			*Pounds.*
644	Wheat (baked) plus wheat straw.	Bull....	79	21	Weak (died).....	22.0
645	.....do.....	...do.....	55	35	.....do.....	20.8
647	Wheat (baked) plus wheat straw plus organic salts.	Heifer...	49	27	Weak (died second day).	26.0
649	.....do.....	Bull....	59	33	Weak (died third day).	13.7
650	Wheat (baked) plus corn stover.	He fer...	54	35	Weak (died after 6 days.)	28.8
651	.....do.....	Bull....	83	16	Strong.....	23.0
637	Wheat flour plus corn stover.	...do.....	88	12	Weak (died in 10 minutes).	No milk.
652	.....do.....	Heifer...	87	7	Strong; lived.....	19.6

The data demonstrated that baking had made no improvement in the whole-wheat grain. The toxic substance present was not destroyed or rendered inocuous by the temperature employed. Further, the variation in the resistance of the individual showed itself in the records of Nos. 650 and 651, as they did with Nos. 637 and 641, whose records are shown in Table III. With corn stover as a roughage, the bad effect of the whole-wheat grain disappeared in some cases, but not in all. Attention should be called to the typical attitude taken by most of the wheat-grain calves, illustrated admirably by the calf of No. 644 (Pl. 26, *D–F*). These animals, unable to stand, would lie on their side with their heads thrown back and respiration labored. Feebly blatting, they would often raise their heads to a central position and then let them fall back again upon their shoulders. When raised to their feet, the animals would make no efforts to stand, and if unsupported would fall into a heap. Practically no use was made of the muscles.

Another important fact to be mentioned in this connection is that a cow, after maturity was reached and growth had ceased, could withstand a wheat and wheat-straw ration very much better than during the growing period. This is also illustrated by cow 644 (Pl. 26, *D–F*), an animal that had received this ration for 12 months. Though these animals became slow in movement and sluggish, yet their coats remained fairly bright and smooth, and to all outward appearances appeared normal. The reproduction records with the flour are in harmony with our other records with the ration of wheat grain and corn stover. For some individuals there was apparently some disturbing factor in the flour, which, by the use of a good roughage like corn stover, was overcome, while with other individuals its effect was to disturb reproduction. However, more data should be accumulated on the disturbing factors in wheat flour before final conclusions can be made. For the present, therefore, this phase of the problem is left open.

INFLUENCE ON REPRODUCTION OF SALTS ADDED TO A CORN RATION

While formulating explanations of our failure with the wheat ration, the theory prominent in our minds was the possibility that the disturbances observed were due to an unfavorable balance of mineral materials or a decidedly acid condition in the animal brought about by a low base supply in the ration. If these were real causal agents, then it should be possible to take a ration known to be physiologically adequate and disturb it by additions of bases or acids, thereby presenting to the cells a new relation of these substances. On this theory it was proposed to add to an all-corn ration such proportions of calcium, magnesium, and potassium as would disturb the relation of base to acid radicals and which, in an all-corn-plant ration, were manifestly adequate for successful growth and reproduction.

Consequently we imposed upon the normal corn ration a mixture of either the carbonates or the organic salts of the bases in the same quantitative make-up as was used with the wheat and wheat-straw ration. The quantity used for 14 pounds of the ration was also identical with that imposed upon a wheat-grain and wheat-straw ration described in an earlier part of this paper, and consisted of 13 gm. of calcium carbonate, 21 gm. of magnesium carbonate, and 51 gm. of potassium carbonate. The proportion of organic salts added was 40 gm. of calcium lactate, 60 gm. of magnesium citrate, and 80 gm. of potassium citrate.

This quantity of bases imposed on a corn ration was arbitrarily chosen, but was probably sufficient in quantity to test adequately the theory proposed. In some cases only magnesium salts were added, but in quantities equivalent to the basicity of the mixture of salts used. There has been suggested many times in both fields of plant and animal nutrition that the relation of calcium to magnesium must be rather definitely proportioned if optimum growth is to be expected. For different plants different calcium-magnesium ratios have been proposed as most favorable, although the entire theory, at least in some of its quantitative aspects, is at present on trial. In animal nutrition it has been suggested that disturbances may arise through an excess of magnesium to calcium in the ration. For example, miller's disease of horses is attributed to the use of wheat bran, and especially to the fact that bran contains an excess of magnesium to calcium. The problem of antagonism of elements and salts is an important one for both plant and animal nutrition, but how far regulating mechanisms at cell surfaces in both plants and animals come into play must always be considered before we adopt the idea of the necessity for optimum behavior of a quantitative relation of the ions or molecules. In the all-corn ration calcium oxid and magnesium oxid stood approximately in the relation of 1 to 1. In the wheat and wheat-straw ration the same relation existed. For purposes of orientation we have imposed upon the corn ration magnesium salts (181 gm. of magnesium citrate per 14 pounds), which would make the calcium-magnesium relation approximately 1 to 2

In Table V these records are shown.

TABLE V.—*Records of reproduction and milk secretion showing influence of additions of salts to an all-corn ration*

No. of cow.	Ration.	Sex.	Weight at birth.	Number of days before time of calving.	Condition of calf.	Milk record (average daily yield for 30 days).
			Pounds.			*Pounds.*
642	Corn ration plus organic salts.	Bull....	75	10	Fairly strong.....	28.0
650	.....do.....	Heifer...	72	5	Strong; lived.....	19.9
575	Corn ration plus carbonates.	Bull....	92	3	.....do.....	28.2
594	.....do.....	...do.....	108	On time	.....do.....	30.7
647	Corn ration plus magnesium citrate.	Heifer...	87	6	.....do.....	34.9
649	.....do.....	Bull....	97	8	.....do.....	25.8

There were no disturbances of an otherwise satisfactory ration by the use of the salts in the quantities here imposed. Even magnesium additions did not disturb reproduction. It is apparent that a considerable range of mineral elements, both as to quantity and quality, may be used in animal nutrition without disturbances, but just what these ranges are must be left for future investigation. It should be recalled that a corn-grain and wheat-straw ration was made efficient by salt additions alone, which would imply that the quantity of base-forming salts in that ration was too low. Milk secretion was also undisturbed by these extra additions of salt to a corn ration. Plate 28 illustrates the effect of these rations on both cows and calves.

INFLUENCE ON REPRODUCTION OF MINERAL ACIDS ADDED TO A CORN RATION

It has been stated that a wheat and wheat-straw ration was of acid character, the urine reacting acid to litmus. For purposes of thorough acquaintance with the influence of such acidity on an otherwise good ration, we added to an all-corn ration daily during gestation such quantities of phosphoric and sulphuric acids as would make the relation of acids to bases approximately similar to that in a wheat and wheat-straw ration. For this purpose there were added to 14 pounds of the all-corn ration 30 c. c. (1.88 sp. gr.) of phosphoric acid and 16 c. c. (1.84 sp. gr.) of sulphuric acid. After dilution with water these were stirred into the corn stover daily, giving it an acid taste not unlike silage. There was no trouble in the continued consumption of this ration. On this diet the urine reacted acid to litmus and showed from 19 to 24 per cent of the nitrogen of the urine as ammoniacal nitrogen, while on an all-corn ration the percentage of nitrogen in the urine as ammonia nitrogen varied from 1.4 to 3.1 per cent of the total nitrogen, and in addition the urine was alkaline to litmus.

That we were accomplishing our purpose of creating an acid ration was apparent; and further, it was clear that this class of animals could aid in maintaining tissue neutrality by the production of ammonia (15).

The records of reproduction are displayed in Table VI.

TABLE VI.—*Records of reproduction and milk secretion on a corn ration plus mineral acids*

No. of cow.	Ration.	Sex.	Weight at birth.	Number of days before time of calving.	Condition of calf.	Milk record (average daily yield for 30 days).
			Pounds.			*Pounds.*
563	Corn ration plus mineral acids.	Bull.....	83	4	Strong.....	19. 1
572	.....do.....	...do.....	78	5	Fairly strong.....	20. 1
563	.....do.....	Heifer...	73	2	Strong.....	19. 9
648	.....do.....	...do.....	77	11	Weak; died.....	23. 9

The results are not entirely harmonious; but in three of the four cases the reproduction was normal, the calves were strong, and they suckled the mother shortly after birth and lived. All of the mothers remained in excellent condition, and we are probably justified in concluding that strong, vigorous mothers would not have reproduction disturbed by an acidity of a ration comparable with that used in this work, provided the ration was otherwise complete. No. 648 did produce a weak calf. This calf made no effort to suckle the mother, could not stand alone, but nevertheless showed none of the head or neck deflections so characteristic of calves produced by wheat-fed mothers. These cows showed no inability to remove the afterbirth, a condition always likely to arise with the premature births on wheat plus wheat-straw rations, unless salt additions had been made. Under the latter circumstances the calf generally would not be carried any nearer to the normal time than where salts were omitted and would either be dead or weak, but the afterbirth would come away normally. This observation is of importance to veterinarians, and the relation of salts in the diet to processes involved in parturition should receive more study.

Illustrations of both calf and mother fed the corn and mineral-acid mixture are shown in Plate 29, *A–B*.

RELATION OF "VITAMINES" TO THE NUTRITION PROBLEM

The discovery that there are at least two essential factors of unknown constitution, both necessary in a ration for growth and probably for reproduction, raised the question as to their relation to these disturbances on an all-wheat-plant ration. As previously stated, these two factors have been designated as "fat-soluble A" and "water-soluble B." We are unquestionably safe in stating that there was a sufficient supply of water-soluble B in our ration of whole wheat plus wheat straw. This

substance is abundantly supplied by the wheat embryo, which constitutes about 5 per cent of the weight of the grain. Fat-soluble A is probably not so abundant in the seeds nor in the particular grain used here—wheat—and for that reason its relation to the problem was given special attention.

The fact that the wheat-grain and corn-stover ration was one giving mixed results, the offspring sometimes being weak and sometimes strong, depending upon the stamina of the mother, suggested its use as one on which the animal could be sensitized. If failure in reproduction should result in a number of cases when butter fat, a good carrier of fat-soluble A, was added to a wheat-grain and corn-stover ration, we would be justified in the conclusion that other factors besides a supply of fat-soluble A and poor ash were at work in causing disaster on a wheat and wheat-straw ration. Consequently butter fat was added to a ration of wheat grain and corn stover at the rate of 2 pounds per hundred of grain. The ration consisted of 6.7 pounds of wheat grain; 0.3 pound of wheat gluten, and 7 pounds of corn stover.

The records of the results on this ration are shown in Table VII.

TABLE VII.—*Records of influence of "vitamines" on reproduction and milk secretion with wheat ration*

No. of cow.	Ration.	Sex.	Weight at birth.	Number of days before time of calving.	Condition of calf.	Milk record. Average daily yield for 30 days.
			Pounds.			*Pounds.*
642	Wheat grain plus corn stover plus butter fat.	Bull.....	44	46	Weak; lived 10 hours.	30.8
653	.....do.....	...do.....	85	13	Strong; lived.....	33.9
653	.....do.....	Heifer....	87	2	Weak; grew strong.	33.0

The data on this phase of the subject support the view that lack of fat-soluble A was not the casual factor in the disturbances recorded. No. 642 on an all-corn and organic-salt ration had produced a strong calf, but on this ration the calf was weak and died (Table VII). The case of No. 653 is interesting and exceedingly important. In her first gestation period on this ration the calf was strong and lived, indicating that the ration was so much improved by the salt additions, through the better roughage used, and a more abundant supply of fat-soluble A as to make possible a successful resistance to the real factor, the toxicity of the wheat kernel.

But this toxicity was apparently cumulative, and in the second gestation period made its effect apparent on the offspring. The calf blatted like a wheat and wheat-straw calf, a weak sort of noise, different from the blat of a normal calf, and would lie flat on the floor, with the head

thrown back, an attitude already described as characteristic for wheat-grain and wheat-straw offspring. On being fed its mother's milk it slowly improved, the symptoms described disappeared, and the animal after one week appeared normal; but only after five days from birth was it able to suckle its mother without help and would have died had it not been given special attention. At the end of two weeks it appeared normal. Plates 29, *C–E*, and 30, *A*, *B*, illustrate the condition of the animals on these rations.

EFFECT OF WHEAT EMBRYO ON REPRODUCTION

It was important that further dissection of the wheat grain be made with the hope of locating in what portion of the kernel toxicity was most prominent. While some data are presented indicating the possibility of toxicity in the flour part of the grain, the work with the embryo makes it clear that it is at least one of the important carriers of toxicity where the whole wheat grain is involved. We have shown that a corn ration was not readily disturbed by moderate shifts in either its bases or acids. Consequently, for purposes of studying the effect of the embryo on reproduction, a basal corn ration was used. We reasoned that should disturbances now arise they could be attributed directly to the imposed wheat embryo. The ration used consisted of seven parts of corn stover, four parts of cornstarch, and three parts of wheat embryo. This ration would introduce from seven to eight times the mass of embryo carried by 8 pounds of whole-wheat meal. In Table VIII the results of wheat-embryo feeding are shown.

TABLE VIII.—*Records of reproduction and milk secretion on wheat embryo*

No. of cow.	Ration.	Sex.	Weight at birth.	Number of days before time of calving.	Condition of calf.	Milk record. Average daily yield for 30 days.
			Pounds.			
562	Cornstarch plus corn stover plus wheat embryo.	Heifer....	24	69	Dead at birth....	No milk.
563	.....do.....	...do.....	32	59	.....do.....	Do.

It is apparent that superimposing the embryo on a good ration, like the corn ration, will bring about early abortion. To all outward appearances the mothers remained in excellent condition, but the calves were notably immature in development and were born over two months ahead of time. We can find no other explanation for these results than that the wheat embryo carried some toxic substance or substances responsible for disturbed growth and reproduction, a result confirmed by the work with swine and rats (4, 5, 12). In Plate 30, *C–D*, the status of one of the mothers and one of the offspring is shown. Note the splendid

condition of the cow. It is apparent that the mature mother may maintain splendid condition, at least for a long time, on a ration which will exhibit its limitations only with the offspring.

CORRECTIVES FOR WHOLE-WHEAT FEEDING WITH THIS CLASS OF ANIMALS

We have made it clear that additions of salt alone will not overcome the toxicity of the wheat-grain and wheat-straw ration. The introduction of corn stover in place of the straw was successful with certain individuals, but often failed with others. By its substitution for the wheat straw the salt mixture was improved, probably a little different protein mixture introduced, and possibly more of fat-soluble A, although it is certain that this change from wheat straw to corn stover did not introduce sufficient of the factors necessary for continued normal nutrition. Our tentative assumption is that the toxicity can be overcome only when either its mass is reduced or there is introduced with a wheat and wheat-straw ration a better protein mixture, a better salt mixture, and an abundance of fat-soluble A. This hypothesis is borne out by the following facts: By substituting casein for the wheat gluten in the ration, thereby shifting the nature of the protein intake and improving its character, both cows, 594 and 654 produced in 1915 strong, vigorous calves weighing 80 and 73 pounds, respectively. The ration consisted of 6.7 pounds of wheat grain, 0.3 pound of casein, and 7 pounds of corn stover. The average daily milk secretion of these animals was 24.6 and 26 pounds, respectively. Plate 30, *E*, shows the condition of No. 654 and her calf of 73 pounds.

When, however, improvement was attempted by additions of only salt and casein, with the straw left in the ration, disaster followed and the calves were either born dead or were weak and died later. This ration consisted of 8.0 pounds of wheat grain, 0.3 pound of casein, 5.7 pounds of wheat straw, and the organic-salt mixture.

In this ration there was a slightly greater amount of toxicity introduced by using a larger proportion of wheat grain, and presumably the straw was contributing but small amounts of fat-soluble A. On this ration cow 655 produced a heifer calf weighing but 49 pounds at birth, 49 days ahead of time and dead, and No. 658 had a male calf of 74 pounds' weight, also dead at birth.

We have, however, had successful results when a part of the wheat straw was displaced by alfalfa hay. Such an addition would theoretically improve the ration through the introduction of a better salt mixture, a different and more efficient protein aggregate, and more of fat-soluble A. These records with alfalfa are shown in Table IX. In addition to the records with wheat grain plus wheat straw plus alfalfa, there are also included records with corn grain plus wheat straw plus alfalfa. The wheat-alfalfa ration consisted of 8 pounds of wheat grain, 0.3 pound of wheat gluten, 2.9 pounds of wheat straw, and 2.9 pounds of alfalfa hay.

The corn-alfalfa ration consisted of 5 pounds of corn grain, 2 pounds of gluten feed, 3 pounds of wheat straw, 3 pounds of alfalfa.

TABLE IX.—*Records of reproduction and milk secretion on a wheat-alfalfa ration*

No. of cow.	Ration.	Sex.	Weight at birth.	Number of days before time of calving.	Condition of calves.	Milk record (average daily yield for 30 days).
			Pounds.			*Pounds.*
642	Corn grain plus wheat straw plus alfalfa.	Heifer...	88	6	Strong; lived.....	26.0
643	.....do.....	...do.....	71	13	.....do.....	27.2
642	.....do.....	...do.....	98	5	.....do.....	35.8
643	.....do.....	...do.....	86	11	.....do.....	39.6
636	Wheat grain plus wheat straw plus alfalfa.	...do.....	61	41	Fairly strong; lived.	No milk.
648	.....do.....	Bull.....	95	1	Strong; lived.....	21.6
648	.....do.....	...do.....	83	2	Fairly strong; lived.	35.0

These records with corn plus wheat straw plus alfalfa show how perfect the ration becomes through the introduction of a natural food material which carries a better mineral content, as illustrated by the substitution of alfalfa hay, with a high mineral content, for wheat straw, with its low mineral supply.

Where the wheat-grain and alfalfa ration was used, the first gestation was fairly successful. Both calves were strong and would have lived independent of the attendant's care. The front legs of the calf of No. 636 were weak.

In the second gestation period, however, the calf of No. 648 was particularly weak in the forelegs and for a period of two to three weeks stood on the first joints. The calf was also blind. It gradually grew strong, but remained permanently blind. This is another illustration of the cumulative effect of the toxicity of the wheat grain. It explains how successfully one may use the whole wheat in a ration carrying a good roughage, or even in such cases how failure may result if the individual mothers are not vigorous and strong or the wheat grain continued in successive gestation periods. It further illustrates the principle that the corrective agents introduced by the alfalfa were better ash, a different and slightly higher protein level, and more of fat-soluble A; it illustrates and emphasizes what profound influences the proper adjustment of the normal factors of nutrition may have on the well-being and the increased resisting powers of the individual even in the presence of the abnormal factor toxicity. Plates 31, and 32, *A–D*, illustrate the results with both corn plus wheat-straw plus alfalfa, and wheat plus wheat-straw plus alfalfa rations.

While failure in reproduction resulted when the ration consisted of corn starch, corn stover, and wheat embryo, it was possible to secure normal offspring when the starch was reduced in quantity and corn meal substituted. On a ration consisting of four parts of corn meal, one part of corn starch, two parts of wheat embryo, and seven parts of corn stover the offspring were apparently normal for the first gestation period. This shows the remarkable supplementary or "antidotal" effect of the corn meal. The mass of embryo in 8 pounds of wheat meal, if we are to attribute all of the deleterious effects on reproduction to the embryo it contains, is about 0.4 pound, and this was the amount that developed disaster in reproduction where wheat was used as the only grain. But in the presence of corn meal at least five times that amount could be used without noticeable disturbance in reproduction, at least in a single gestation. Plate 32, *E*, illustrates this result.

GENERAL DISCUSSION OF RESULTS

We should first like to emphasize the fact that all individuals whose records are involved in this discussion were free from contagious abortion. The herd had been under the observation of Dr. F. B. Hadley, professor of veterinary science in the Experiment Station, during the entire period of experimentation.

In our attempts to locate the trouble in the all-wheat ration (wheat grain plus wheat straw) we have fed rations made up of corn grain and wheat straw. Here the offspring were weak and were often born dead. When to that same ration, however, a suitable salt mixture was added, so that the ash content of the ration was like that of the all-corn ration, perfect offspring resulted. This would clearly indicate that one of the deficiencies of an all-wheat-plant ration was a proper salt mixture. When, however, the corn grain in the above ration was displaced by the wheat grain and the ration consisted of wheat grain plus wheat straw and salts, disaster again resulted, which showed the presence of another disturbing factor in the wheat grain. Calves born by mothers upon this ration showed peculiar deflections of the head, inability to get up and suckle the mother, and in most cases have died within a few hours after birth.

These experiments indicate that in the all-wheat-plant ration there were two factors operative against normal nutrition—namely, a poor salt mixture and inherent toxicity of the wheat grain. When the wheat grain was coupled with corn stover, we have sometimes met with success and sometimes with failure in the character of the offspring. With strong mothers it appears that the corn stover may become an "antidote" and thereby furnish sufficient of all the normal factors of nutrition so as to enable the animal to reproduce normally.

The possibility of destroying the toxicity by heat was also investigated, and baked wheat was fed with corn stover. This had no effect whatever in improving the wheat kernel.

In other cases the wheat-grain and corn-stover ration had butter fat added to it for the purpose of supplying plentifully the growth-promoting factor, fat-soluble A, now known to be necessary for growth and supplied abundantly in butter fat. It was thought possible that the wheat-grain and wheat-straw ration was somewhat deficient in this material. Additions of butter fat, however, did not uniformly improve the ration. We had a number of failures in reproduction, and also a number of successes with its use. This would again emphasize the probability of the presence of a toxic substance in the wheat grain.

When, however, the wheat grain was mixed with a legume hay, such as alfalfa, so that the latter formed but 20 per cent of the ration, we have had perfect success in all cases in the production of normal offspring, at least for the first gestation. The improvement resulting from the use of the alfalfa must lie in introducing in the ration a better salt mixture, perhaps a better protein mixture, and an abundance of growth-promoting substances, all of which may contribute toward making it possible for the cell to destroy or resist the action of the toxic substance introduced. However, in the second-gestation period on the same ration (wheat grain plus wheat straw plus alfalfa hay) the calves were weak, and in one case blind, but it lived. This is extremely interesting as illustrating the cumulative effect of this toxicity.

Where corn stover was wholly substituted for the wheat straw, we had a number of successes and also a number of failures in the first gestation period. Apparently as an "antidote" to the toxicity this roughage was not as effective as the legume hay.

We had thought it possible in our earlier work that the acidity of the wheat ration was an important factor in the results recorded. It was true that the urine of the all-wheat-plant-fed animals showed a slight acidity to litmus, owing to a low intake of bases in the ration. If this were an important factor in our results, then the successful corn ration might be disturbed with acids and give us results similar to the wheat ration. This, however, we found not to be the case, for when to an all-corn ration there were added mineral acids such as sulphuric and phosphoric acids in such proportions as to make the acidity of the urine of a degree similar to that of a wheat and wheat-straw-fed animal, the offspring were strong and normal in every respect. Even the addition of a high proportion of magnesium salts to a corn ration did not disturb in any way its power of producing normal offspring.

The results detailed above indicate clearly that wheat grain contains a toxic material, and later work has shown that this is very prominent in the embryo of the seed. When wheat embryo is imposed on corn stover so as to bring into the ration seven to eight times the amount of em-

bryo that would be introduced when feeding whole wheat, the result is likely to be an early abortion. The calf is now dropped at six to eight months; this demonstrates that the increased mass of the toxic material produces this disturbance at a somewhat more rapid rate.

This result was particularly likely to occur where no other grain was used with the embryo. With both corn meal and corn stover in the ration the detrimental effect of the wheat embryo was nullified, at least for a single gestation period.

It is an interesting fact that in the wheat milling industry the embryo passes into wheat bran in small amounts but in much greater quantities in wheat middlings. The wheat flour that is used for bread making has the least content of embryo of any of the wheat by-products.

In an attempt to obtain an anatomical picture of the condition responsible for the physiological disturbances as already described, Dr. Bunting, of the medical school of the University of Wisconsin, kindly consented to make a histological study of the tissues from a number of the abnormal calves. In general, no striking lesions were revealed. Livers and kidneys showed some degeneration (hydropic) changes, but the nervous tissues gave the most evidence of the presence of an excessive amount of fluid, a condition of edema. This histological picture was analogous to that of beriberi, the result of feeding polished rice, and it also simulated, if it was not identical, with that obtained from the spinal cord of pigs on certain rations as described in a previous publication (5). The edema was observed between the membranes covering the cord, around the blood vessels, and around the nerve cells. In these instances the nerve cell and their nuclei were shrunken, the latter staining more intensely than normally. No abnormalities in medullation of the fibers of the cord as demonstrable by the Weigert stain were observed. While the observations did not point to anything especially characteristic, it is probable that the motor disturbances observed in the animals can be referred to the edematous condition of the nervous tissues.

The cause of beriberi is ascribed to the absence or deficiency of certain essential factors in the diet, particularly to water-soluble B. In the case of excessive wheat feeding it would appear that the essential causal factor for disaster to growth and reproduction is a toxic substance which either interferes with the utilization of materials necessary for the full development of the nervous system of the animal or directly with the normal functioning of this tissue. This would account for the blindness observed in some of the heifers and also for the failure of muscular coordination apparent in the new-born calves produced on rations of large whole-wheat content.

It was also apparent that rations producing an early delivery of offspring would usually lead to a failure of the animal to remove properly the afterbirth, with its attending dangers of infection; and an

overabundance of a material like wheat straw in a ration, owing to its low salt content, becomes an important factor in premature birth.

An observation in our experimental work of interest to veterinarians was the low resistance to other diseases of the mothers fed the wheat ration. In an outbreak of anthrax in the university herd the only losses to occur from this diease in our experimental herd were among the wheat-grain fed animals.

The principle (5, 12) laid down as to what factors must be present in a ration of natural origin in order that it become efficient for both growth and reproduction is well supported by these data. This principle postulates that there must be present efficient proteins, adequate energy, proper salt mixture, fat-soluble A and water-soluble B (vitamines) and an absence of toxicity, or a toxicity of such mildness as to become inocuous in the presence of the other normal factors of nutrition. The presence of toxicity in the wheat kernel as the explanatory factor for these records rests not only upon the evidence secured with swine and rats but also on that presented here. It is not a deficiency phenomenon. A corn-grain and corn-stover ration is physiologically adequate, while a wheat-grain and corn-stover ration often failed not only when used alone but when there was added to it the most likely limiting factor, fat-soluble A, as butter fat.

The recognition of these normal factors of nutrition and the further recognition of the occurrence in apparently normal foodstuffs of substances of mild toxicity will be of immense advantage in arriving at an understanding of the oft-reported troubles with farm animals, which to-day are either not understood or their etiology is wrongly assigned; and in the field of human nutrition the same principles will apply.

When a few years ago the corn crop of Nebraska failed to mature because of drought, and early rains had produced a bumper wheat crop, it left many farmers with little to feed their breeding stock but wheat grain and certain roughages. In many cases where this was done the calves were born either dead or weak, with great financial losses to many breeders. No one would have suspected that the ration was a factor in these disasters, but it undoubtedly was the direct cause of the trouble.

When Dakota farmers, with their only roughage as wheat straw, try to build up an aninal-husbandry industry, there is likely to arise trouble in reproduction with this class of animals, unless other roughages with better salt mixtures are brought into the ration. We are informed that there is already much trouble with reproduction by cows in the Dakotas wherever much wheat straw is fed. Such facts as these must emphasize the importance of an understanding of all the factors of animal nutrition and in addition an understanding of all the factors contributed by any particular foodstuff. It should further emphasize how such studies can furnish the facts which will aid the animal feeder in avoiding the danger zones of his art. We need more effort placed on the accu-

mulation of information on the physiological behavior of feeding stuffs than on the attempts to bring out new mathematical expressions of feeding standards.

These experiments further show the limitations of the theory of a "balanced" ration as now expressed and indicate the very great importance of other factors besides protein and energy in the successful diet. It was indeed surprising to find that the common wheat kernel had a low toxicity; but such factors as toxicity, growth-promoting substances of unknown nature, proper balance of salts, indicate how complex the problems of animal nutrition really are and how necessary it is that these factors be clearly exposed in order that we may place the various feeds in their proper category. We have pointed out how a material of low toxicity, such as the wheat kernel, may be used with success. A good roughage like a legume hay was an admirable "antidote." Even corn meal and a poorer roughage like corn stover served to offset the detrimental effects of a large mass of wheat embryo. This also illustrates how an adjustment of the normal factors of nutrition may conceal the presence of the detrimental factors.

It is important to keep constantly in mind that the disclosure of either a nutritive deficiency or the presence of an abnormal factor in a common natural foodstuff should not necessarily condemn its use. It should, however, emphasize the need of combining it in the ration with those other natural products which will either supply abundantly the deficiencies or act as an "antidote" to any inherent toxicity.

SUMMARY

This paper summarizes the results of further studies of the physiological value of restricted rations. The data presented are limited to observations on growing and reproducing heifers and are especially concerned with the effect of the nutrients from the wheat and corn plants.

Restriction to the wheat plant as a source of "balanced" nutrients (wheat grain plus wheat straw) did not sustain growth with Holstein heifers. Such animals also failed to show œstrus and could not be bred.

Marked pathological conditions resulted, such as blindness, feeble and emaciated condition, and abnormal excitability followed by collapse. Evidence is presented which fixes the responsibility for such a condition on two factors in the ration: (1) poor salt mixture and (2) inherent toxicity in the grain.

Improvement of the ration could not be made by additions of salt alone.

By the use of corn stover as a roughage in place of the wheat straw, growth was sustained but reproduction was only partially successful, dependent upon the stamina of the mother. Where reproduction was

successful in the first gestation period, it failed in the second, owing to the cumulative effect of the wheat toxicity.

By the use of alfalfa hay to take the place of one-half of the wheat straw, results similar to those with corn stover were secured. Growth was splendid, reproduction normal in the first gestation period, but weakness appeared in the second gestation. The alfalfa and corn stover introduced a better salt mixture, a little different protein mixture, and probably a more plentiful supply of growth-promoting substances, all of which, according to our hypothesis, would either individually or collectively improve the ration, but not necessarily make it perfect. It might still fail if the mass of toxicity was too large.

Baking the wheat grain did not improve it.

The particular effect of these all-wheat-grain rations was to cause marked histological changes in the nervous tissue of the offspring. The motor cells partly degenerated and the spinal cord showed more or less edematous condition. This was analogous to our observations on swine with wheat-grain feeding. On wheat-grain and wheat-straw rations growing heifers also showed symptoms of nerve degeneration, as evidenced by blindness and great excitability. The causes of the disturbance were due to the inherent toxicity of the wheat grain and not to "deficiencies of vitamines."

Corn grain plus wheat straw allowed sustained growth, but at a slow rate. The offspring were weak or dead. Additions of salt to this ration made it normal, indicating that this was the only factor needed for perfect nutrition with this ration.

A physiologically complete ration such as the corn-grain and corn-stover mixture could not be disturbed, at least in a single gestation, by altering the calcium-magnesium ratio through the addition of magnesium salts. Even the addition of mineral acids to this ration, in such quantities as to make the urine of the individuals receiving it acid to litmus and rich in ammonium salts, did not disturb its nutritive completeness.

The addition, however, of wheat embryo to a corn ration did cause disturbances, bringing about early abortions. This was due to its high content of the toxic material of the wheat kernel.

Considerations of the influence of such investigations on practice are also presented.

LITERATURE CITED

(1) FUNK, Casimir.
1913. STUDIEN ÜBER DAS WACHSTUM. I. MITTEILUNG. DAS WACHSTUM AUF VITAMINHALTIGER UND VITAMINFREIER NAHRUNG. *In* Ztschr. Physiol. Chem., Bd. 88, Helf 5, p. 352–356. Literatur, p. 356.

(2) ——— and MACALLUM, A. B.
1914. DIE CHEMISCHEN DETERMINANTEN DES WACHSTUMS. *In* Ztschr. Physiol. Chem., Bd. 92, Heft 1, p. 13–20, pl. 2. Literatur, p. 20.

(3) HART, E. B., and HUMPHREY, G. C.
1915. THE RELATION OF THE QUALITY OF PROTEINS TO MILK PRODUCTION. *In* Jour. Biol. Chem., v. 21, no. 2, p. 239–253, 4 charts.

(4) ——— and MCCOLLUM, E. V.
1914. INFLUENCE ON GROWTH OF RATIONS RESTRICTED TO THE CORN OR WHEAT GRAIN. *In* Jour. Biol. Chem., v. 19, no. 3, p. 373–395, 11 charts, 1 pl.

(5) ——— MILLER, W. S., and MCCOLLUM, E. V.
1916. FURTHER STUDIES ON THE NUTRITIVE DEFICIENCIES OF WHEAT AND GRAIN MIXTURES AND THE PATHOLOGICAL CONDITIONS PRODUCED IN SWINE BY THEIR USE. *In* Jour. Biol. Chem., v. 25, no. 2, p. 239–259, 9 fig., 5 pl.

(6) ——— MCCOLLUM, E. V., STEENBOCK, H., and HUMPHREY, G. C.
1911. PHYSIOLOGICAL EFFECT ON GROWTH AND REPRODUCTION OF RATIONS BALANCED FROM RESTRICTED SOURCES. Wis. Agr. Exp. Sta. Research Bul. 17, p. 131–205, 24 fig.

(7) HOPKINS, F. C.
1912. FEEDING EXPERIMENTS ILLUSTRATING THE IMPORTANCE OF ACCESSORY FACTORS IN NORMAL DIETARIES. *In* Jour. Physiol., v. 44, no. 5/6, p. 425–460, 7 fig.

(8) MCCOLLUM, E. V.
1914. THE VALUE OF THE PROTEINS OF THE CEREAL GRAINS AND OF MILK FOR GROWTH IN THE PIG AND THE INFLUENCE OF THE PLANE OF PROTEIN INTAKE ON GROWTH. *In* Jour. Biol. Chem., v. 19, no. 3, p. 323–333.

(9) ——— and DAVIS, Marguerite.
1913. THE NECESSITY OF CERTAIN LIPINS IN THE DIET DURING GROWTH. *In* Jour. Biol. Chem., v. 15, no. 1, p. 167–175, 5 charts.

(10) ——— ———
1915. THE ESSENTIAL FACTORS IN THE DIET DURING GROWTH. *In* Jour. Biol. Chem., v. 23, no. 1, p. 231–246, 12 charts.

(11) ——— and KENNEDY, Cornelia.
1916. THE DIETARY FACTORS OPERATING IN THE PRODUCTION OF POLYNEURITIS. *In* Jour. Biol. Chem., v. 24, no. 4, p. 491–502.

(12) ——— SIMMONDS, Nina, and PITZ, Walter.
1916. THE NATURE OF THE DIETARY DEFICIENCIES OF THE WHEAT EMBRYO. *In* Jour. Biol. Chem., v. 25, no. 1, p. 105–131, 19 charts.

(13) OSBORNE, T. B., and MENDEL, L. B.
1913. THE RELATION OF GROWTH TO THE CHEMICAL CONSTITUENTS OF THE DIET. *In* Jour. Biol. Chem., v. 15, no. 2, p. 311–326, 7 charts.

(14) ——— ———
1914. AMINO-ACIDS IN NUTRITION AND GROWTH. *In* Jour. Biol. Chem., v. 17, no. 3, p. 325–349, 8 charts.

(15) STEENBOCK, H., NELSON, V. E., and HART, E. B.
1914. ACIDOSIS IN OMNIVORA AND HERBIVORA AND ITS RELATION TO PROTEIN STORAGE. *In* Jour. Biol. Chem., v. 19, no. 3, p. 399–419.

(16) STEPP, Wilhelm.
1911. EXPERIMENTELLE UNTERSUCHUNGEN ÜBER DIE BEDEUTUNG DER LIPOIDE FÜR DIE ERNÄHRUNG. *In* Ztschr. Biol., Bd. 57, Heft. 5, p. 135–170.

(17) ———
1913. FORTGESETZTE UNTERSUCHUNGEN ÜBER DIE UNENTBEHRLICHKEIT DER LIPOIDE FÜR DAS LEBEN ... *In* Ztschr. Biol., Bd. 62, Heft 9/10, p. 405–417.

PLATE 18

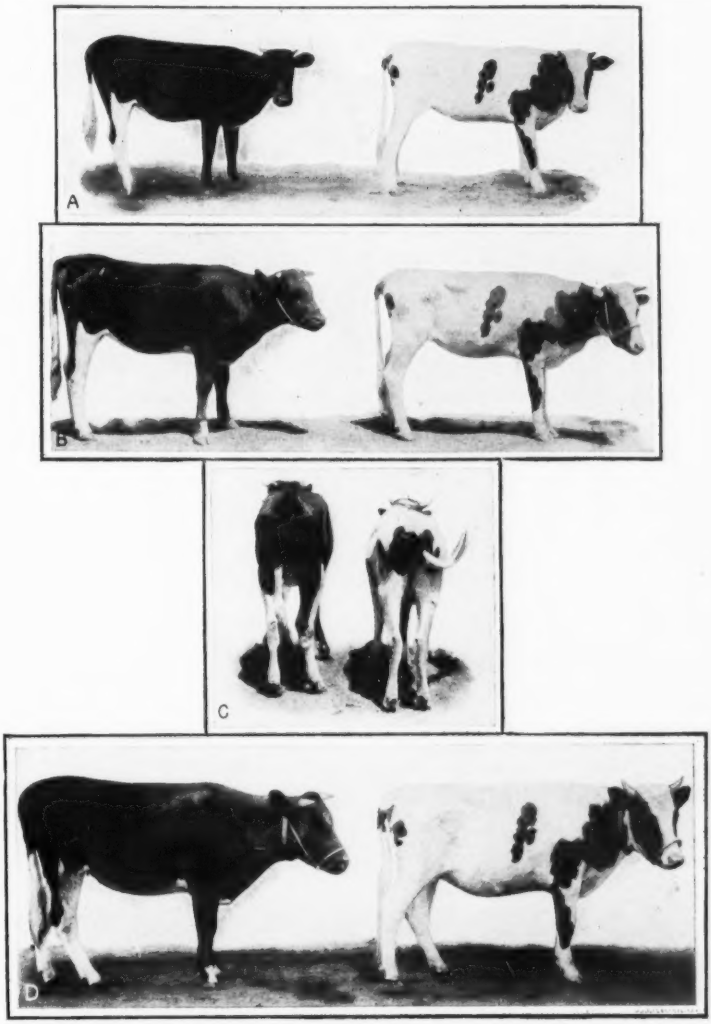
Cattle showing the effect of a ration of wheat grain and wheat straw:

A.—Condition at the initiation of the experiment. June, 1910.

B.—Condition after 12 months on the ration. Note the sluggish and sleepy condition. June, 1911.

C.—Condition after 12 months on the ration. Note the distinct emaciation. June, 1911.

D.—Condition of two yearlings after 18 months on the ration. Both are in sluggish condition and one of them is blind. December, 1911.


A
B
C
D

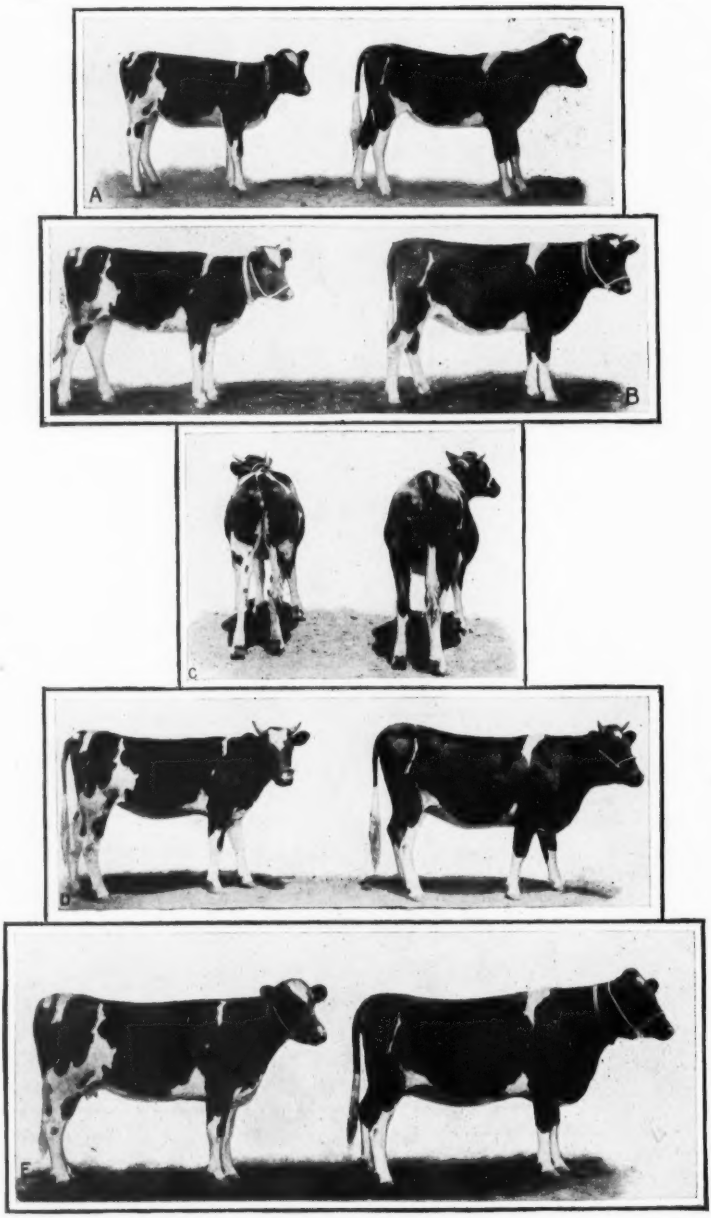

A
B
C
D
E

PLATE 19

Cattle showing the effect of a ration of corn grain and corn stover:

A.—Condition at the initiation of the experiment. June, 1910.

B.—Condition after 12 months on the ration. Note the alert and thrifty condition. June, 1911.

C-D.—Condition after 12 months on the ration. June, 1911.

E.—Condition after 30 months on the ration. Note the splendid condition. November, 1912.

98975°—17——4

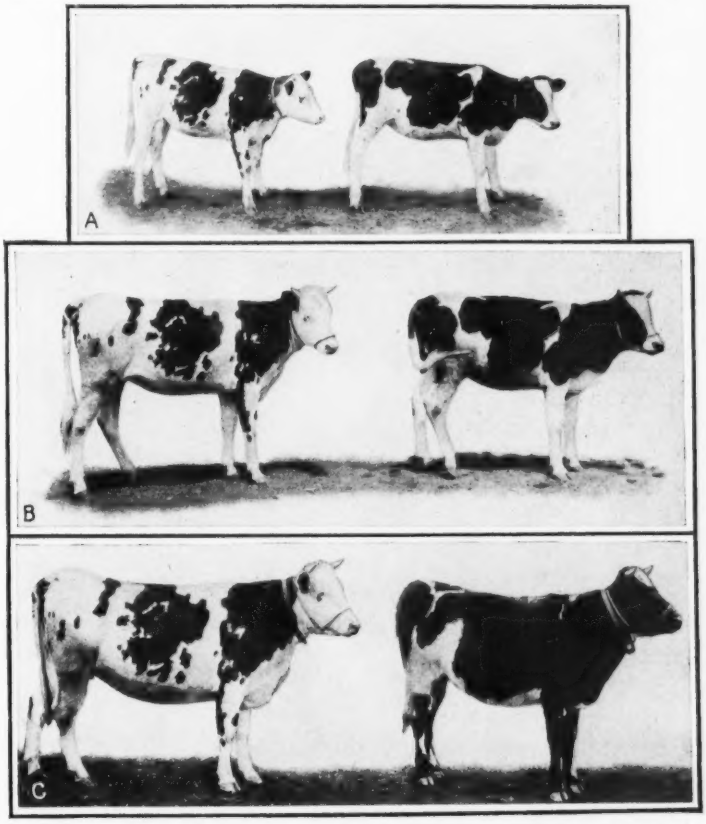
PLATE 20

Cattle showing the effect of a ration of corn grain and wheat straw:

A.—Condition at the initiation of the experiment. June, 1910.

B.—Condition after 12 months on the ration. Note the rather poor condition of these individuals; poor growth had resulted. June, 1911.

C.—Condition after 30 months on the ration. Poor growth had been made and only a fair condition maintained. December, 1912.


A
B
C

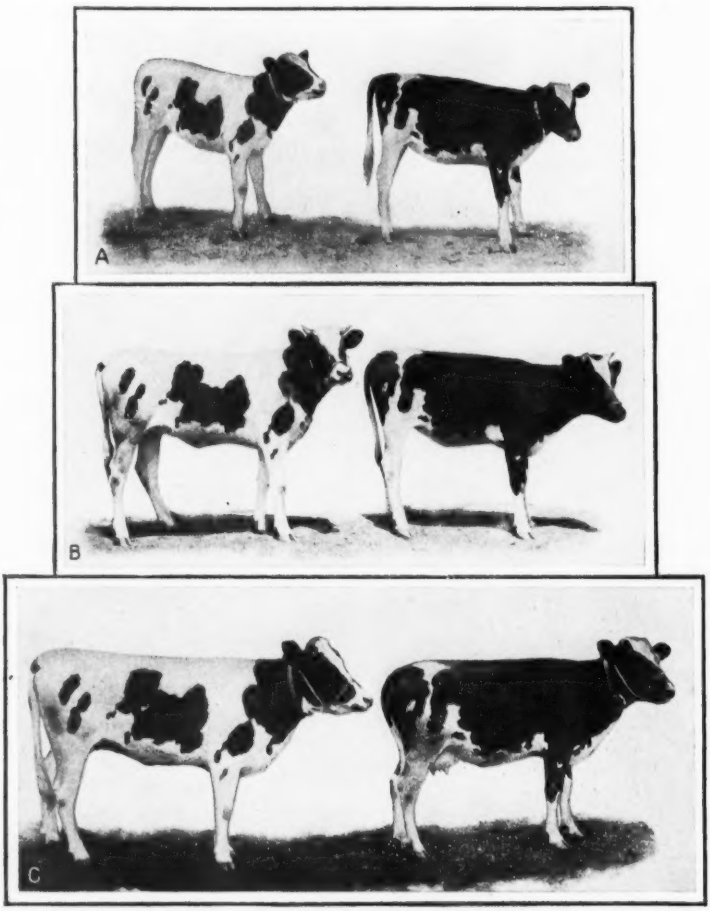

A
B
C

PLATE 21

Cattle showing the effect of a ration of wheat grain and corn stover:

A.—Condition at the initiation of the experiment. June, 1910.

B.—Condition after 12 months on the ration. Growth was below normal, although the animals were in fair condition. June, 1911.

C.—Condition after 30 months on the ration. Growth below normal. Condition only fair. November, 1912.

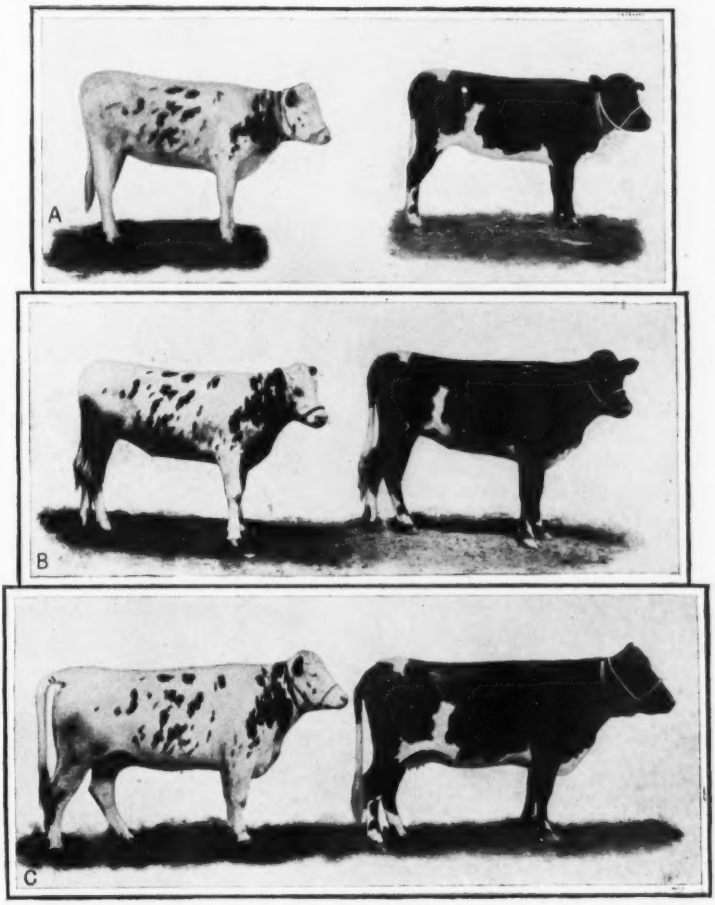
PLATE 22

Cattle showing the effect of a ration of corn grain, wheat straw, and alfalfa:

A.—Condition at the initiation of the experiment. November, 1910.

B.—Condition after 6 months on the ration. In good condition and growing well. June, 1911.

C.—Condition after 24 months on the ration. In splendid condition. November, 1912.


A
B
C

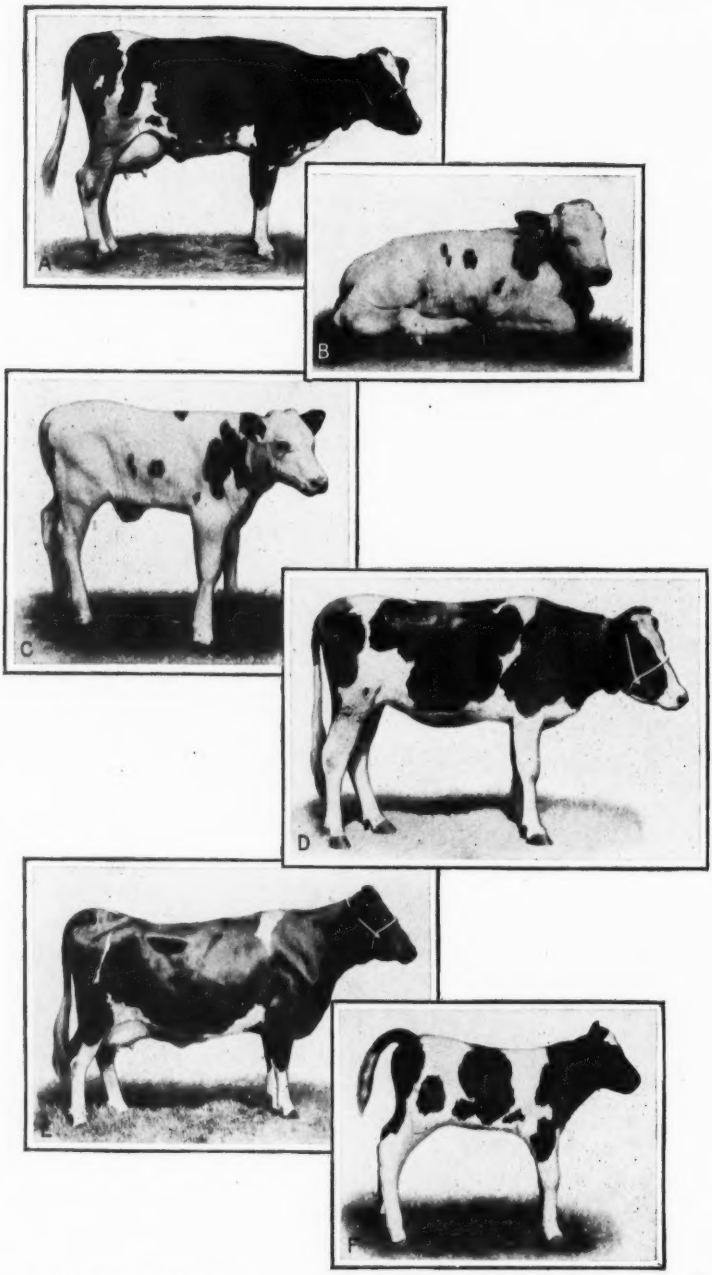

A
B
C
D
F

PLATE 23

Cattle showing the effect of a ration of wheat grain and corn stover:

A–C.—*A*, Mother (No. 637) in poor condition. *B*, Calf weak with peculiar attitude of head. *C*, Calf grew strong.

D.—A heifer (No. 635) showing the effect of a ration of corn grain and wheat straw after 24 months. Note the sleepy and unthrifty condition. She would not breed.

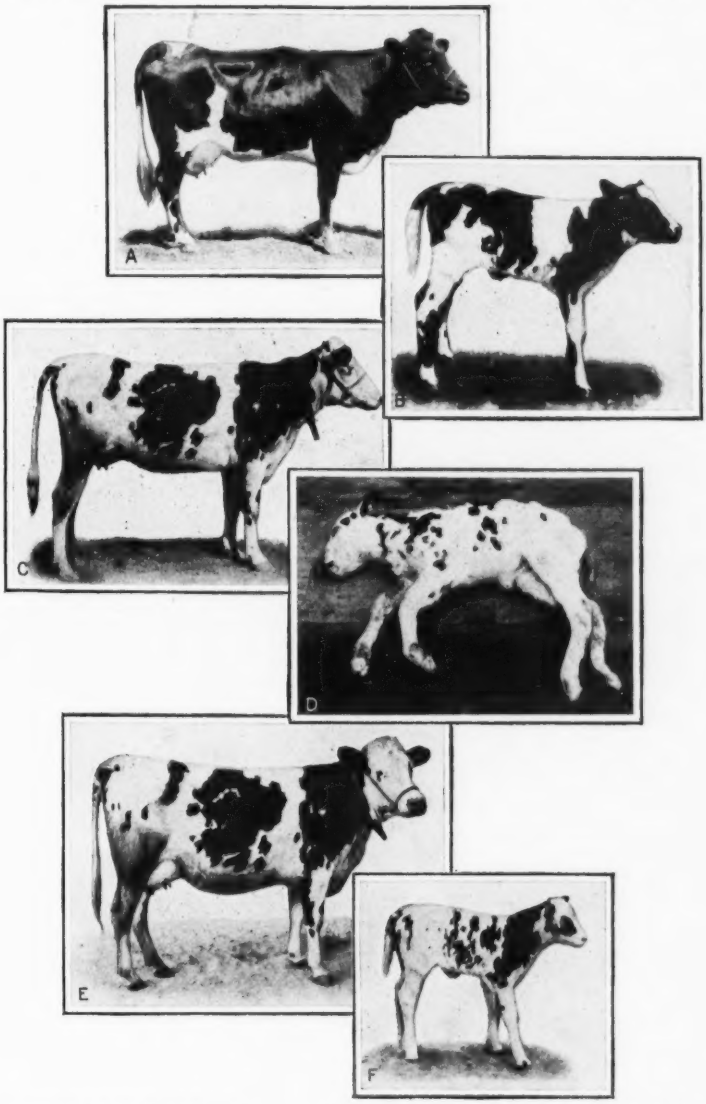
E–F.—A cow (No. 575) and her calf, showing the effect of an all-corn-plant ration after 24 months. They were in splendid condition.

PLATE 24

A–B.—A cow (No. 642) and her calf, showing the effect of a ration of corn grain, wheat straw, and alfalfa hay. The addition of the alfalfa made growth possible and reproduction normal.

C–D.—A cow (No. 636) and her calf, showing the effect of a ration of corn grain and wheat straw. The result of a poor salt mixture. Growth was fair, but the mother was in poor condition and dead offspring resulted.

E–F.—A cow (No. 636) and her calf, showing the effect of a ration of corn grain, wheat straw, and organic salts. By the addition of the salts the mother's condition not only improved, but normal calves resulted.


A
B
C
D
E
F

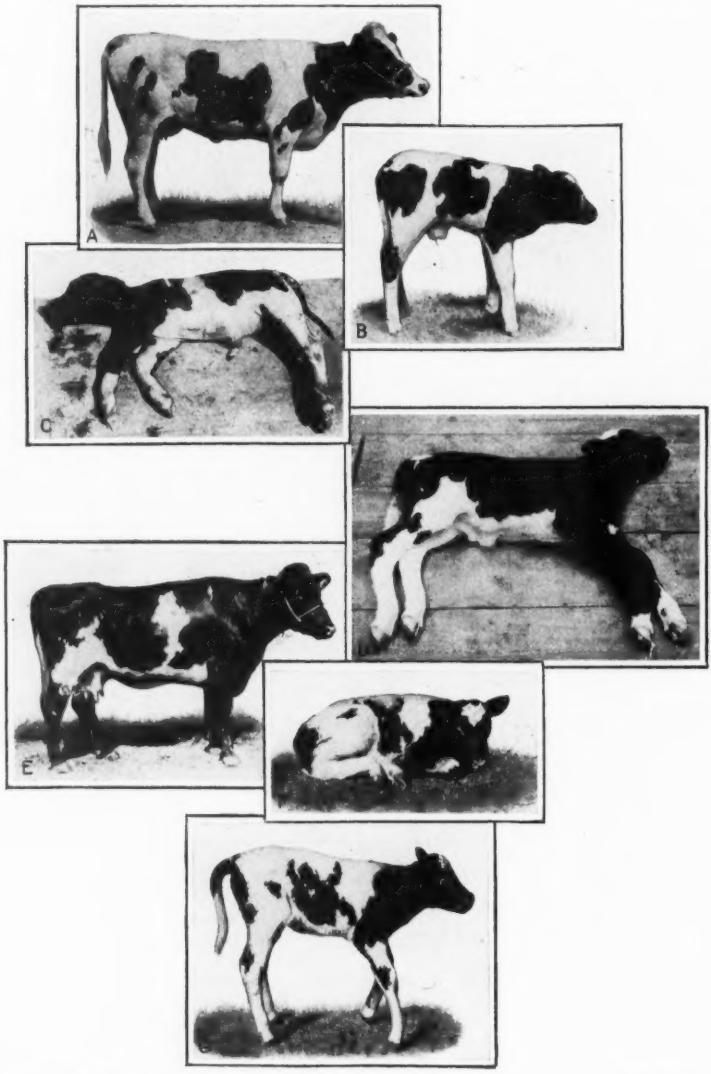

A
B
C
E

PLATE 25

A–B.—A cow (No. 641) and her calf, showing the effect of a wheat grain and corn-stover ration. The mother was in fair condition and the offspring was fairly strong. Contrast with Plate 23, *A*, *B*. This indicates how this roughage (corn stover) in some cases adequately supplements the wheat grain, but was not successful with all individuals.

C.—The calf of cow 641, showing the effect of a ration of wheat grain, wheat straw, and magnesium citrate. An illstration of an unsuccessful attempt to improve the wheat ration with a magnesium salt. In no case could it be done. The calf was dead and immature.

D.—The calf of cow 651, showing the effect of a wheat-grain, wheat-straw, and organic-salt mixture. No mixture of salts alone made the ration a normal one for reproduction. This calf was born 35 days ahead of time, weak, and died soon after birth. Note the tendency to throw the head back, a characteristic attitude taken by such animals when alive.

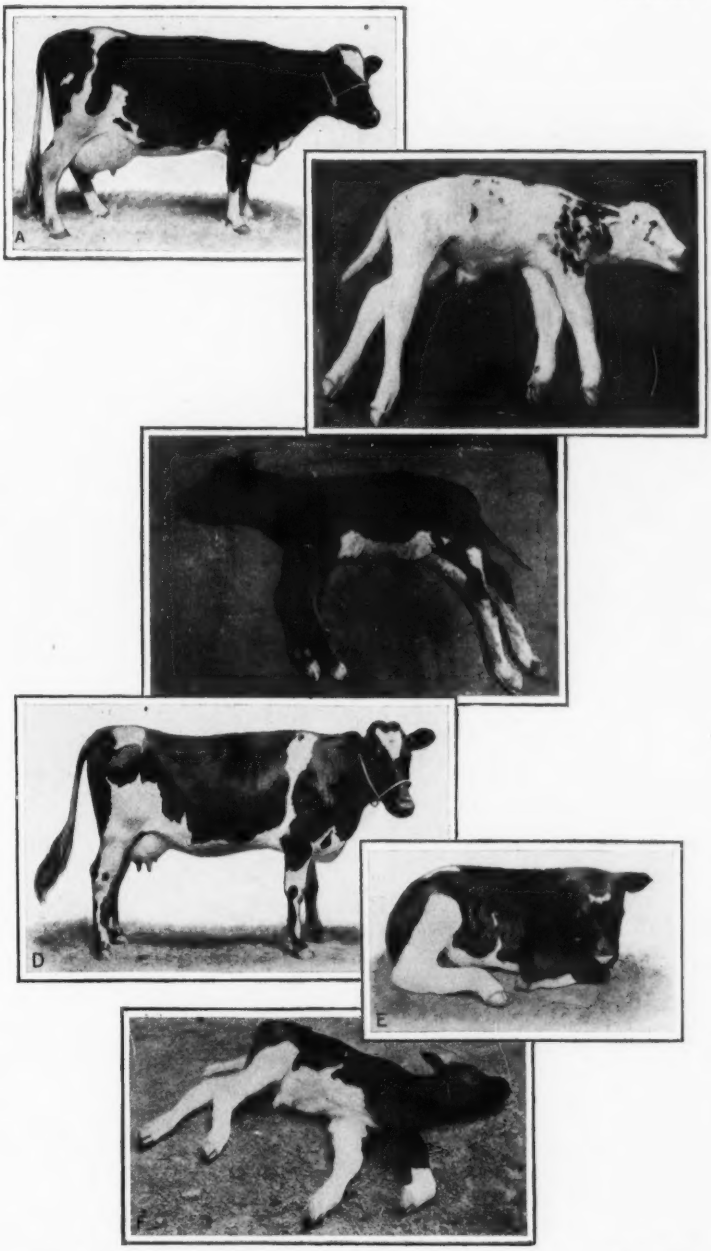
E–G.—A cow (No. 570) and her calf, showing the effect of a wheat-grain, wheat-straw, and organic-salt mixture. This shows the possibility of maintaining the mother in apparently fair condition by additions of a salt, but dead or weak offspring result. This calf lived three days. It had a peculiar blat, entirely unlike normal calves.

PLATE 26

A–B.—A cow (No. 637) and her calf, showing the effect of a wheat-grain and corn-stover ration. Another illustration of the impossibility of general success with corn stover as a supplement to the wheat grain. The mother appeared in good condition, but the offspring was dead.

C.—The calf of cow 636, showing the effect of a wheat-grain, wheat-straw, and organic-salt mixture. The toxicity of the wheat grain could not be overcome by the salt mixture alone. Contrast with Plate 24, *E*, *F*, where the same cow received the same ration, with corn grain instead of wheat grain.

D–F.—A cow (No. 644), showing the effect of a ration of baked wheat and wheat straw. The mother was in fair condition. The calf was alive when photographed, but was weak and could not stand. Note the deflection of the head, a characteristic posture when lying flat, as shown in figure E. The calf died. Baking had not improved the grain.


A
D
E
F

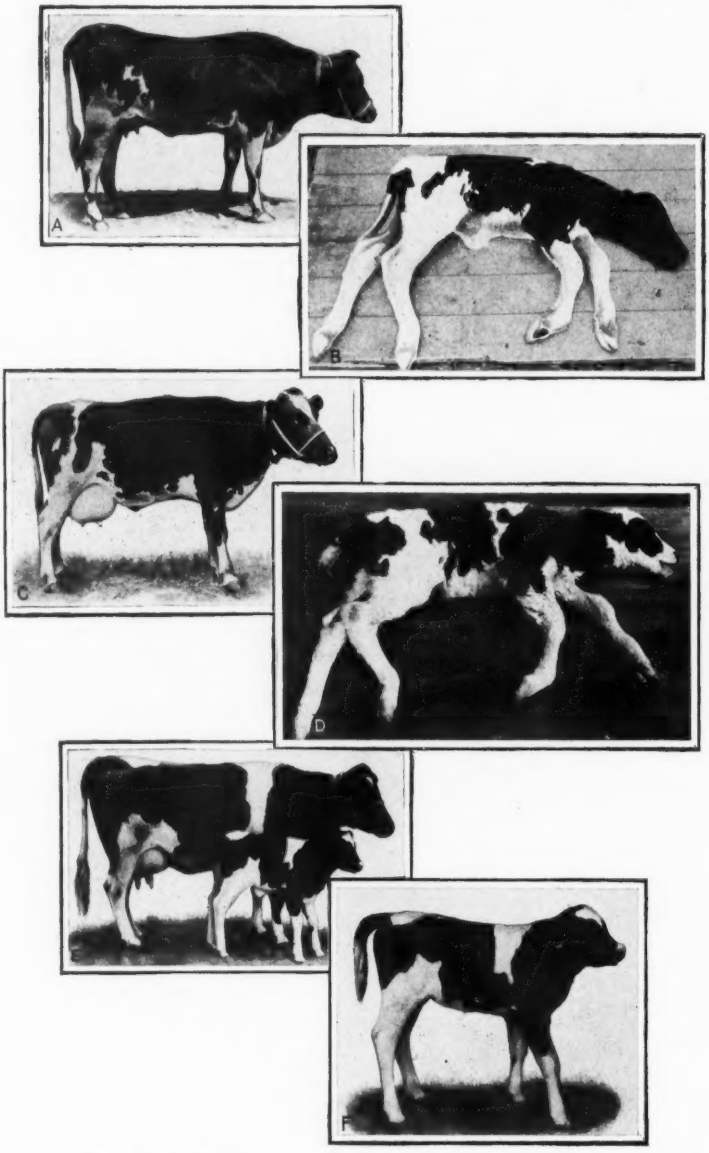

A
B
C
D
F

PLATE 27

A–B.—A cow (No. 645) and her calf, showing the effect of a baked-wheat, wheat-straw, and organic-salt mixture. The calf was dead at birth. Baking and salt additions had not made the ration a perfect one.

C–D.—A cow (No. 637) and her calf, showing the effect of wheat-flour and corn-stover ration. With this cow this ration was unsuccessful for reproduction.

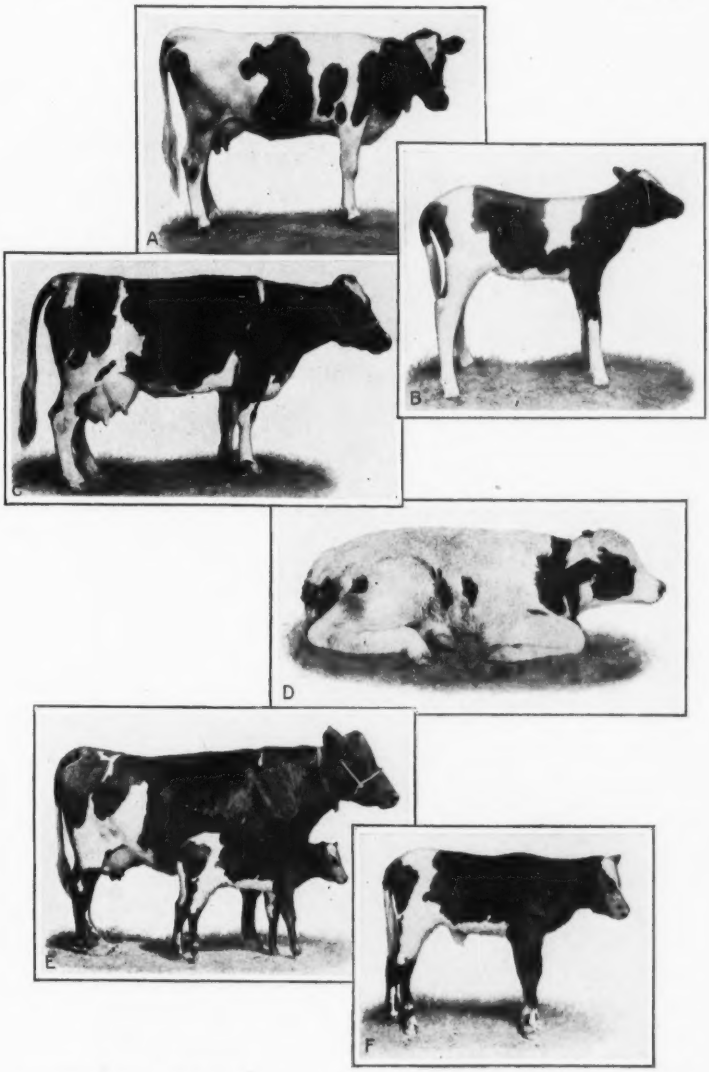
E–F.—A cow (No. 652) and her calf, showing the effect of a wheat-flour and corn-stover ration. With this cow this ration was successful in reproduction. These figures illustrate the differences in individual resistance and make it probable that even wheat flour carries some toxicity, which could not be successfully overcome by corn stover, at least with all individuals.

PLATE 28

A–B.—A cow (No. 650) and her calf, showing the effect of a ration of corn grain, corn stover, and organic salts. The physiological soundness of an all-corn plantation was not disturbed by altering the relation of base and acid radicles. Both calf and mother appeared perfectly normal.

C–D. A cow (No. 594) and her calf, showing the effect of a ration of corn grain, corn stover, and a mixture of inorganic bases as carbonates. The calf was strong and weighed 108 pounds when born. The efficiency of the ration was not destroyed by a change in the relation of bases to acids.

E–F.—A cow (No. 647) and her calf, showing the effect of a ration of corn grain, corn stover, and magnesium citrate. Even additions of magnesium salts alone in quantities sufficient to make the ratio of calcium to magnesium 1 to 2 did not destroy the efficiency for reproduction of an otherwise good ration. The mother and calf were strong and apparently normal.


A
B
C
D
E
F

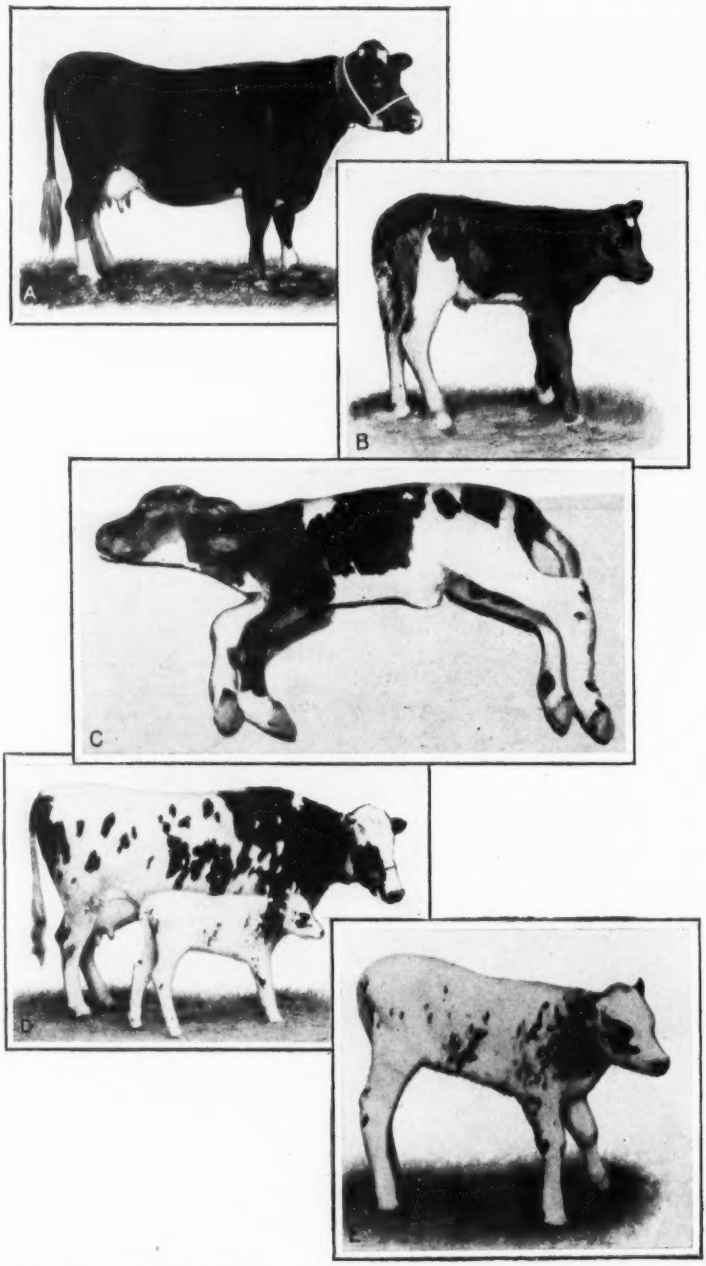

A
B
C
D

PLATE 29

A–B.—A cow (No. 563) and her calf, showing the effect of a ration of corn grain, corn stover, sulphuric acid, and phosphoric acid. The ration was acid, and the urine produced was acid to litmus. The ammonia production in the urine was very high, yet the ration was physiologically perfect, and normal, strong offspring resulted. Note the good condition of both mother and calf.

C.—The calf of cow 642, showing the effect of a ration of wheat grain, wheat gluten, corn stover, and butter fat. A generous supply of fat-soluble A was not alone sufficient to make a ration rich in wheat grain always successful for reproduction. Such results as this make it clear that disaster with the wheat grain was not due to a deficiency but to a toxicity. The calf was born dead.

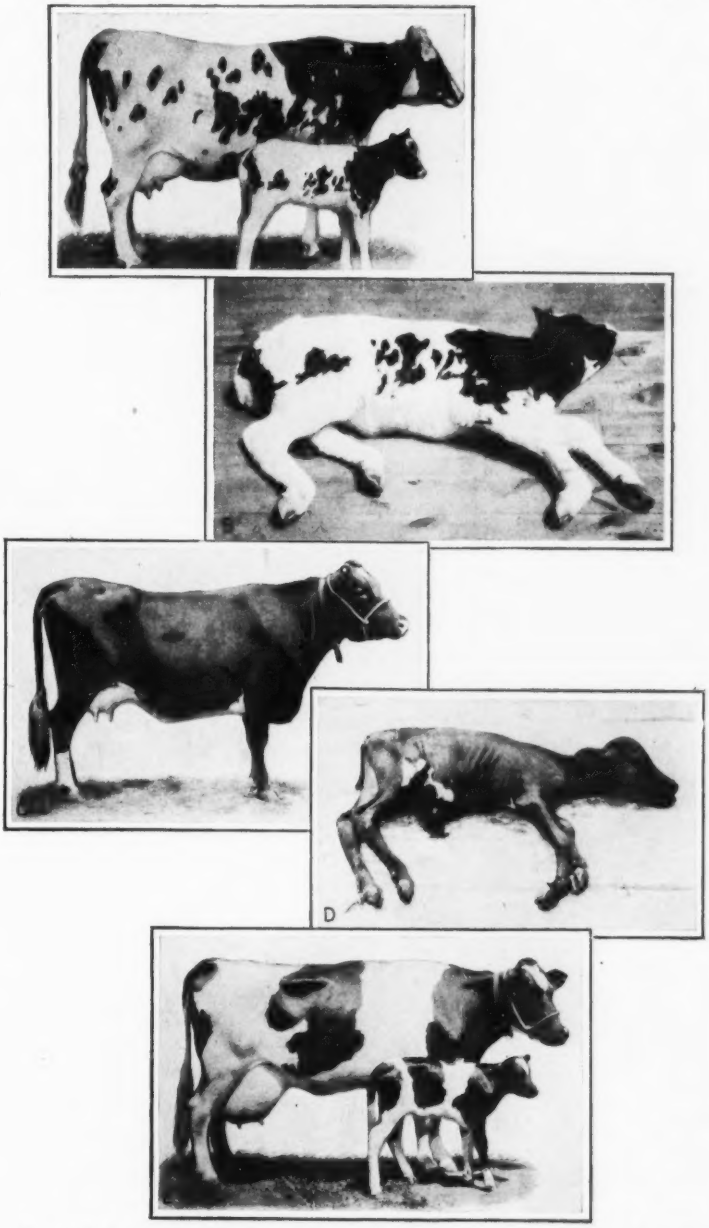
D–E.—A cow (No. 653) and her calf, showing the effect in 1915 of a ration of wheat grain, wheat gluten, corn stover, and butter fat. In this case successful reproduction resulted; but the fact that without the butter fat the ration was often successful illustrates the principle of individual resistance to the wheat toxicity, at least for a time. The calf was born strong. (See Pl. 24, *A–B*.)

PLATE 30

A–B.—The same cow (No. 653) shown in Plate 29, *D*, illustrating the effect in 1916 of the same ration as in 1915. This calf was born weak (fig. *B*). Note the deflection of the head and neck. It was unable to stand. Photographed a few hours after birth. Given the mother's milk it gradually improved, as shown in figure *A*. This illustrates how continuation of this ration over a second gestation period may weaken the offspring. The toxicity of the wheat grain was cumulative in effect.

C–D.—A cow (No. 562) and her calf, showing the effect of a ration of wheat embryo, corn starch, and corn stover. The embryo of the wheat grain carried a considerable mass of toxicity. Massing this in the ration brought on early abortion, with a gestation period of six to seven months. Note the very good general appearance of the mother.

E.—A cow (No. 654) and her calf, showing the effect of a ration of wheat grain, casein, and corn stover. This illustrates how by the improvement of the salt mixture of a ration and probably by introducing an abundance of the growth-promoting substances through the substitution of corn stover for wheat straw, and in addition by improving the proteins of the ration by the use of casein the wheat grain toxicity can be overcome. Both cow and calf were strong and vigorous.


D

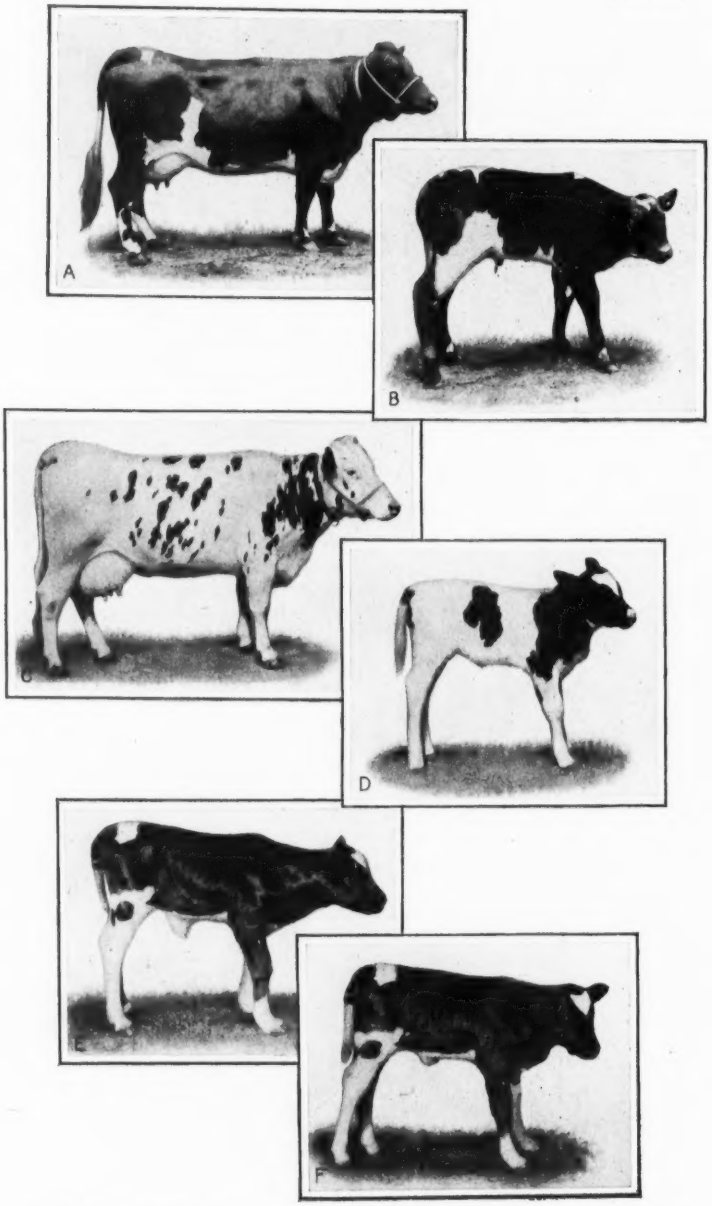

A
B
C
D
E
F

PLATE 31

A-B.—Cow 642 and her calf, showing the effect of a ration of corn grain, wheat straw, and alfalfa hay. Without the alfalfa hay disaster in reproduction would have resulted. The calf was normal and vigorous.

C-D.—Cow 643 and her calf, showing the effect of a ration of corn grain, wheat straw, and alfafa hay. Without the alfalfa hay disaster in reproduction would have resulted. The calf was normal and vigorous.

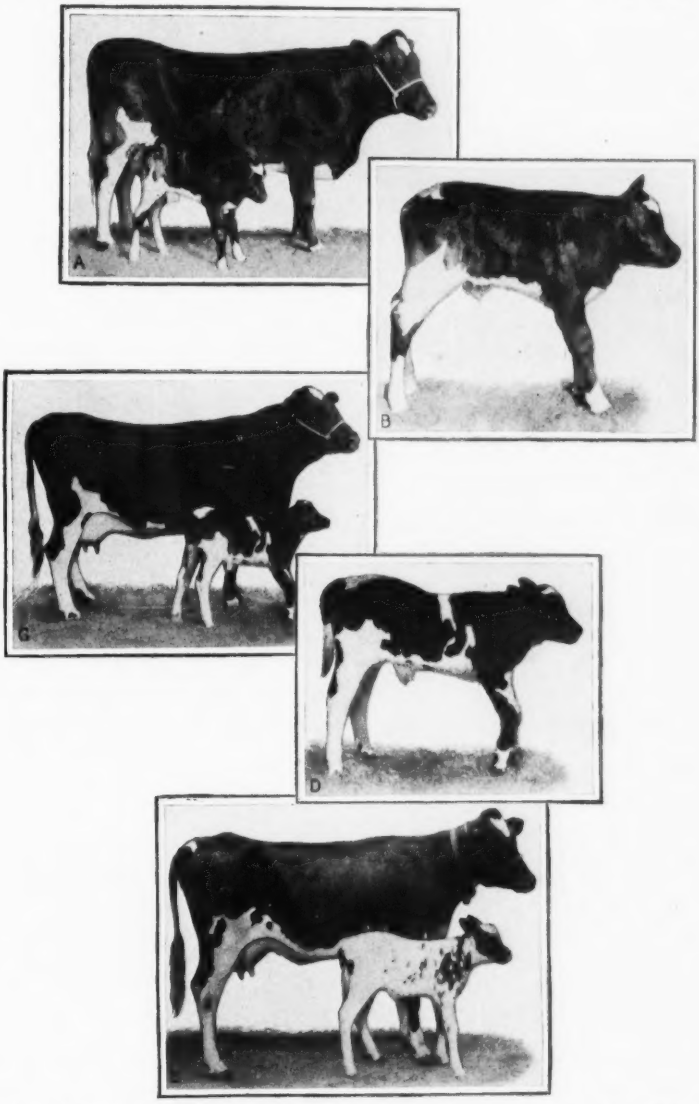
E-F.—The calf of cow 636, showing the effect of a ration of wheat grain, wheat straw, and alfalfa hay. The introduction of half of the roughage as alfalfa hay made the ration a success; at least for a single gestation period. The probable factors introduced were better ash, different proteins, and more of the growth-promoting substances fat-soluble A and water-soluble B. These factors enabled the animal to resist the effects of toxicity. The calf was strong.

PLATE 32

A–B.—A cow (No. 648) and her calf, showing the effect in 1915 of a ration of wheat grain, wheat straw, and alfalfa hay. With half of the roughage as alfalfa hay reproduction in the first gestation period was successful. The calf was normal and strong, and the cow was apparently healthy and vigorous.

C–D.—The same cow (No. 648), showing the effect in 1916 of the second gestation on the same ration, wheat grain, wheat straw, and alfalfa hay. This calf was carried to full time, but was weak and at first was fed from the bottle. It grew strong, but the fore legs were so weak that it stood for the first few days of its life on the first joints. This calf was blind. The mother remained in apparently good condition. This again illustrates the cumulative effect of wheat toxicity.

E.—A cow (No. 662) and her calf, showing the effect of a ration of corn meal, starch, wheat embryo, and corn stover. Successful reproduction in the presence of the embryo. At least for the first gestation the "antidotal" properties of corn meal and corn stover were sufficient to overcome the toxic effects of the wheat embryo. Without the corn meal and with only wheat embryo, starch, and corn stover in the ration reproduction would have been premature and the calf either dead or markedly undersized. (See Pl. 30, *D*.)


A
B
G
D

TOXIC VALUES AND KILLING EFFICIENCY OF THE ARSENATES

By A. L. LOVETT, *Entomologist*, and R. H. ROBINSON, *Assistant Chemist*,
Oregon Agricultural Experiment Station

INTRODUCTION

Investigations covering a period of years have been under way at the Oregon Agricultural Experiment Station to determine the killing efficiency of various poison sprays for insects. More recently the problem has resolved itself into a consideration of certain definite arsenical sprays, their relative values as insecticides, and a determination of the probable effective dilution for practical horticultural spraying.

The present paper deals with the results obtained in a study of the relative toxic value of pure samples of lead hydrogen arsenate (acid), basic lead arsenate (neutral), and calcium arsenate. A preliminary study of the relative toxicity of the arsenates of lead was made by Tartar and Wilson,[1] of this Station. The present report on the lead arsenates is the continuation of that work on an enlarged scale, which, in addition to affording further verification of their results, gives material data on the following points: (1) The comparative time required to kill small caterpillars and nearly mature caterpillars; (2) the approximate amount of lead hydrogen arsenate and basic lead arsenate required to kill small caterpillars and nearly mature caterpillars; (3) the proportion of these arsenates devoured by the small and mature caterpillars that passes through the alimentary canal of the larvæ.

In addition to similar comparative tests of the calcium arsenate, it was desired to determine the burning tendency on apple foliage (*Malus sylvestris*) in the field.

EXPERIMENTAL PROCEDURE

Our common tent caterpillar (*Malacosoma pluvialis* Stretch) was used throughout the whole series of experiments. The caterpillars were collected in the field almost wholly from the wild rose (*Rosa nukatana* Presl.). A few tents were obtained from the apple and some from *Crataegus* sp. In collecting the caterpillars the limb to which the tent was secured was severed and brought in, the caterpillars being disturbed as little as possible. All the larvæ used in one set or series of tests were collected

[1] TARTAR, H. V., and WILSON, H. F. THE TOXIC VALUES OF THE ARSENATES OF LEAD. *In* Jour. Econ. Ent., v. 8, no. 5, p. 481-486. 1915.

Journal of Agricultural Research,
Washington, D. C.
jh

Vol. X, No. 4
July 23, 1917
Key No. Oreg.—1

the same day. The caterpillars varied considerably in size, particularly in the tests with the larger forms. Every reasonable effort was made to eliminate errors which might result from such variations in size or in the vitality. To accomplish this, the numerous tents and accompanying foliage were in some cases left piled together overnight. Most of the caterpillars would then collect on some prominent branch, and thus a very uniform mixing would be obtained; otherwise, care was taken to secure this uniformity when introducihg the caterpillars to the sprayed foliage.

In all cases the solutions were applied to the foliage with an ordinary 1-quart glass-jar hand sprayer, care being taken to keep the material agitated and to get the solution thoroughly applied and evenly distributed. The solution was allowed to dry on the foliage before introducing the caterpillars. The specimens of sprayed foliage were transferred to vessels containing water, so that the foliage would remain fresh and attractive. Approximately 1,000 caterpillars were used in each test. From time to time a few would drop from the foliage and crawl away. These were always discarded.

Every day, Sundays excepted, following the introduction of the caterpillars, the dead larvæ (drop) were collected from the oilcloth spread under the vessels containing the foliage. The twig was first jarred sharply to dislodge any dead caterpillars that might be caught in the web. The caterpillars were picked up one at a time, carefully brushed with a camel's-hair brush, and placed in a paper sack, which was labeled with the test number, date, and total drop for the day. The excrement was then collected and run through a fine soil sieve to remove any bits of foreign material. Pieces of leaves, molted skins, etc., were carefully removed. The excrement was then placed in glass vials, properly labeled, and filed for analysis, after which the oilcloth was washed and dried.

The arsenates used in the experiments were prepared by one of us, in order that as pure a compound as possible could be assured. Lead hydrogen arsenate, basic lead arsenate, and calcium arsenate were employed. The lead arsenates were prepared as directed by Robinson and Tartar,[1] while the calcium arsenate, which is a salt of variable composition, was prepared by a method recently devised at this Station. It gave upon analysis the following composition:

	Per cent.
Calcium oxid (CaO), total	28.14
Arsenic pentoxid (As_2O_5), total	57.91
Water (H_2O), by difference	13.95

This approximates closely the theoretical composition of $CaH.AsO_4$.

[1] Robinson, R. H., and Tartar, H. V. The arsenates of lead. Ore. Agr. Exp. Sta. Bul. 128, 32 p., 3 fig. 1915.

EXPERIMENTAL WORK

SERIES A.—VERY SMALL CATERPILLARS

The caterpillars employed in series A were the very small ones and varied in size from those just hatched to forms about 5 to 7 days old (approximately 10 mm. in length). Both wild-rose and apple foliage was used. As the caterpillars were collected mostly on the rose, it was considered possible that for a time they might refuse to eat apple foliage and then feed to excess when they did begin. Since lead arsenate and water alone failed to stick well or spread evenly on the rose foliage, flour paste (1 pound of flour to 1 gallon of water) was prepared. This gallon of paste was diluted with 3 gallons of water and used at the rate of 20 c. c. to the 1,000 c. c. of lead-arsenate solution. No flour paste was added to the lead-arsenate solution on the apple foliage.

The strength of solutions used, date of application, and daily drop are recorded in Table I.

TABLE I.—*Effect of arsenates on very small caterpillars*

Kind and strength of solution.	Foliage.	Date sprayed.	Drop (April).														
			9	10	11	12	13	14	15	16	17	18	19	20	21	22	Total.
Lead hydrogen arsenate (acid) (2:50).	Rose..	Apr. 8	48	65	92	449	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	654
Do.....	Apple.	8	17	19	[a]752	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	788
Basic lead arsenate (neutral) (2:50).	Rose..	8	39	155	181	141	720	.....	.....	.....	.....	.....	.....	.....	.....	.....	1,236
Do.....	Apple.	8	5	22	163	83	522	.....	.....	.....	.....	.....	.....	.....	.....	.....	795
Calcium arsenate (2:50).	Rose..	8	41	101	202	63	659	.....	.....	.....	.....	.....	.....	.....	.....	.....	1,066
Do.....	Apple.	8	28	116	75	69	752	.....	.....	.....	.....	.....	.....	.....	.....	.....	1,040
Lead hydrogen arsenate (acid) (1:50).	Rose..	8	49	84	160	1,264	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1,557
Do.....	Apple.	8	27	54	88	859	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1,028
Basic lead arsenate (neutral) (1:50).	Rose..	8	15	43	100	71	729	.....	.....	.....	.....	.....	.....	.....	.....	.....	958
Do.....	Apple.	8	23	131	243	253	51	807	....	.....	.....	.....	.....	.....	.....	.....	1,508
Lead hydrogen arsenate (acid) (1:400).	Rose..	8	....	7	24	74	259	298	518	.....	.....	.....	.....	.....	.....	.....	1,180
Do.....	Apple.	8	....	7	62	236	424	1,151	.....	.....	.....	.....	.....	.....	.....	.....	1,880
Basic lead arsenate (neutral) (1:400).	Rose..	8	....	4	6	23	39	63	36	....	[b]104	....	119	....	165	[a]342	901
Do.....	Apple.	8	....	4	3	1	15	4	0	....	[b]101	....	320	.....	.....	[a]1,035	1,483
Lead hydrogen arsenate (acid) (1:800).	Rose..	9	....	.....	9	124	49	33	[b]59	....	111	121	[b]27	....	140	.....	673
Do.....	Apple.	9	.....	.....	16	45	0	195	[b]113	....	179	37	[b]13	....	312	.....	910
Lead hydrogen arsenate (acid) (1:1,200).	Rose..	9	.....	.....	21	93	247	313	[b]157	....	390	156	[b]47	.....	.....	[a]342	1,766
Do.....	Apple.	9	.....	.....	18	23	47	98	[b]54	....	126	44	[b]24	.....	.....	[a]310	744
Control.....	Rose..	8	.....	.....	.....	.....	(c)	.....	.....	.....	(c)	.....	.....	(c)	.....	.....	.....

[a] Very few; 15 to 20 yet on foliage show feeble signs of life.
[b] New foliage prepared and added.
[c] Discontinued.

SERIES B.—NEARLY MATURE CATERPILLARS

Series B was similar in every way to series A except that the caterpillars used were from half-grown to nearly mature larvæ and only apple foliage was used. The results are given in Table II.

TABLE II.—*Effect of arsenate on nearly mature larvæ*

Kind and strength of solution.	Date sprayed.	Drop (May).															Total.
		5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
	May.																
Lead hydrogen arsenate (acid) (2:50)	4	40	39	....	264	286	....	....	....	....	....	....	....	....	....	....	629
Basic lead arsenate (neutral) (2:50)	4	....	....	....	121	287	....	46	....	....	....	....	....	....	....	....	454
Calcium arsenate (2:50)	4	188	194	....	113	221	....	....	....	....	....	....	....	....	....	....	716
Lead hydrogen arsenate (acid) (1:50)	4	38	373	....	392	217	....	....	....	....	....	....	....	....	....	....	1,020
Basic lead arsenate (neutral) (1:50)	4	....	....	....	87	234	125	43	....	....	....	....	....	....	....	....	489
Calcium arsenate (1:50)	4	136	371	....	334	241	....	....	....	....	....	....	....	....	....	....	1,082
Lead hydrogen arsenate (acid) (1:100)	5	....	82	....	483	331	....	98	....	....	....	....	....	....	....	....	994
Lead hydrogen arsenate (acid) (1:400)	5	....	6	....	22	163	162	[a]151	426	107	136	....	....	....	....	....	1,173
Calcium arsenate (1:400)	4	....	73	....	481	397	116	124	....	....	....	....	....	....	....	....	1,191
Lead hydrogen arsenate (acid) (1:800)	5	....	12	....	34	123	164	[a]203	279	108	....	....	201	....	....	....	1,112
Calcium arsenate (1:800)	4	....	10	....	152	370	191	117	....	....	....	....	....	....	....	....	850
Lead hydrogen arsenate (acid) (1:1,200)	5	....	7	....	9	16	23 (a)	[a]33	145	187	....	153 (a)	[a]103	....	318 (b)	39	1,026
Control	6	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....

[a] New foliage prepared and added. [b] Two parasitized.

DISCUSSION OF RESULTS

The results of the tests to determine the relative killing efficiency of the hydrogen and basic lead arsenate as shown by data contained in the above tables (I and II) and summarized in Table IV approximate very closely those obtained by Tartar and Wilson[1] in 1915 and substantiate in general the work of previous investigators, in that the acid arsenate has a decidedly higher killing efficiency at a given dilution than does the neutral arsenate. It will be noted that the figures in the tables do not emphasize the differences in killing efficiency of these arsenates, particularly with the more concentrated solutions, but observation during the actual tests showed the differences clearly. Had the drop been recorded more frequently than every 24 hours, this difference, as indicated in the tables, would probably have been more marked. From Tables II and IV it is seen that until the fourth day no caterpillars died on the twigs sprayed with neutral lead arsenate, while both the acid lead

[1] TARTAR, H. V., and WILSON, H. F. Op. cit.

arsenate and calcium arsenate were very effective toxic agents on the first day. In regard to rapidity of action and killing efficiency, calcium arsenate and acid lead arsenate were approximately the same.

A comparison of the relative time required to kill the small larvæ and the nearly mature caterpillars, as indicated in Table IV, shows that although there was a higher percentage of the latter dropped in the early days of the test, it required a longer period to kill all of the larger forms.

CHEMICAL ANALYSIS

After the collection and drying of the poisoned caterpillars and excrement from the various tests, the arsenic content was determined. The method used is as follows: A counted number of caterpillars, or excrement therefrom, were accurately weighed and introduced into a Kjeldahl flask (500 c.c. capacity) together with about 15 c.c. of arsenic-free concentrated sulphuric acid. The flask was then placed over a free flame and arsenic-free nitric acid added in small quantities at short intervals until all of the organic matter was oxidized and the solution was perfectly clear. The solution was allowed to digest over a hot flame for an hour, when all the nitric acid was expelled and white fumes of sulphuric acid (H_2SO_4) were given off. Following the digestion, the excess of sulphuric acid was driven off and the arsenic determined by titration with *N/100* iodin solution after reduction with potassium iodid according to the modified Gooch and Browning[1] method.

For convenience in making comparisons, the results of the chemical analysis are calculated in milligrams contained in the amount of sample taken and also the equivalent amount in 1 gm. of dried sample. Table III gives the data obtained.

These results throughout show consistently a higher arsenic content in the tissue and a lower arsenic content in the corresponding excrement with lead hydrogen arsenate as the spray than with either the basic lead arsenate or calcium arsenate. Calcium arsenate comes second in this comparison, while the basic lead arsenate contained the smallest amount of arsenic in the tissue and shows that a large amount passed through the intestinal tract unchanged, most of the arsenic eaten being found in the excrement.

A further study of the ratio of arsenic in the tissue to that contained in the excrement brings out some very interesting facts. In Tables III and IV we note for lead hydrogen arsenate (2:50) that there was found 0.90 mgm. of arsenic pentoxid per gram of tissue, as compared with 0.49 mgm. of arsenic pentoxid per gram of excrement, or a ratio of 1 to 0.544; in the calcium arsenate (2:50) there was 0.83 mgm. per gram of tissue

[1] WILEY, H. W., ed. OFFICIAL AND PROVISIONAL METHODS OF ANALYSIS, ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, AS COMPILED BY THE COMMITTEE ON REVISION OF METHODS. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), p. 239. 1908. Reprinted in 1912.

and 0.64 mgm. in the excrement, or a ratio of 1 to 0.70; in the basic lead arsenate (2:50) there was 0.35 mgm. per gram of tissue and 0.53 mgm. in the excrement, or a ratio of 1 to 1.51. This ratio varies somewhat in the weaker strengths, but is consistent throughout, in that the amounts of arsenic are higher in the tissue and lower in the excrement in the relative order as noted above.

TABLE III.—*Arsenic consumed by caterpillars*

VERY SMALL CATERPILLARS

Kind and strength of solution.	Number of caterpillars.	Dry caterpillars.			Dry excrement.		
		Weight.	Arsenic pentoxid.		Weight.	Arsenic pentoxid.	
			Total.	Per gram of tissue.		Total.	Per gram of excrement.
		Gm.	*Mgm.*	*Mgm.*	*Gm.*	*Mgm.*	*Mgm.*
Lead hydrogen arsenate (acid) (2:50)	1,442	0.3130	0.23	0.74	0.0200	0.058	0.28
Lead hydrogen arsenate (acid) (1:400)	3,060	1.0625	.17	.19	.3700	.086	.23
Lead hydrogen arsenate (acid) (1:800)	1,583	.7200	.12	.18	.3400	.086	.25
Lead hydrogen arsenate (acid) (1:1,200)	2,510	.9870	.14	.15	.4500	.12	.25
Basic lead arsenate (neutral) (2:50)	2,031	.5500	.17	.31	.0900	.065	.72
Basic lead arsenate (neutral) (1:50)	2,466	.5920	.23	.39	.0650	.058	.89
Basic lead arsenate (neutral) (1:400)	2,384	1.6520	.29	.17	1.3400	.17	.13
Calcium arsenate (2:50)	2,106	.5800	.23	.40	.....	.....	.....

NEARLY MATURE CATERPILLARS

Lead hydrogen arsenate (acid) (2:50)	383	5.7350	5.10	0.90	0.4700	0.23	0.49
Lead hydrogen arsenate (acid) (1:50)	803	4.3025	2.19	.51	.6300	.23	.36
Lead hydrogen arsenate (acid) (1:100)	565	4.7832	1.73	.36	1.1200	.12	.11
Lead hydrogen arsenate (acid) (1:400)	586	5.0720	.64	.13	2.0100	.17	.09
Lead hydrogen arsenate (acid) (1:800)	556	5.0540	.63	.12	2.8150	.23	.08
Lead hydrogen arsenate (acid) (1:1,200)	513	4.2080	.63	.16	2.8840	.23	.08
Basic lead arsenate (neutral) (2:50)	287	3.2800	1.15	.35	1.4030	.75	.53
Basic lead arsenate (neutral) (1:50)	455	3.7950	1.04	.28	1.6300	.58	.35
Calcium arsenate (2:50)	185	3.8950	3.22	.83	.5400	.34	.64
Calcium arsenate (1:50)	705	5.8760	1.90	.32	1.0480	.46	.44
Calcium arsenate (1:400)	532	4.6900	.63	.14	2.2700	.35	.15
Calcium arsenate (1:800)	152	1.4200	.17	.12	3.9783	.40	.11

This may be explained as being due to the relative stability of the three salts, since lead hydrogen arsenate and calcium arsenate are comparatively unstable compounds and would probably react more readily with the body juices, permitting a large amount of the arsenic to be assimilated and therefore retained within the tissue. The basic lead arsenate, on the other hand, is a very stable salt and would probably pass through the body comparatively unchanged, so that more arsenic would be found in the excrement than in the tissue.

TABLE IV.—*Comparative results of tests to determine the relative killing efficiency of arsenates*

Kind and strength of solution.	Number of days after being introduced that first caterpillars died.		Percentage of caterpillars dead first day of drop.		Total number of days to kill.		Approximate amount of total arsenic devoured per 1,000 caterpillars.		Ratio of arsenic pentoxid in tissue is to arsenic pentoxid in excrement.	
	Small caterpillars.	Mature caterpillars.	Small caterpillars.	Mature caterpillars.	Small caterpillars.	Mature caterpillars.	Small caterpillars.	Mature caterpillars.	Small caterpillars.	Mature caterpillars.
							Mgm.	*Mgm.*		
Lead hydrogen arsenate (2:50)	0	0	4.5	6.36	4	5	.....	11.815	.....	1:0.5440
Lead hydrogen arsenate (1:50)	0	0	2.94	2.74	4	5	.....	7.395	.....	1:.7058
Lead hydrogen arsenate (1:100)	.....	0	.....	8.25	.....	6	.....	3.995	.....	1:.3055
Lead hydrogen arsenate (1:400)	1	0	.46	.50	7	9	.1595	1.870	1:1.2100	1:.0900
Lead hydrogen arsenic (1:800)	1	0	1.58	1.08	12	8	.1633	1.700	1:1.3888	1:.6660
Lead hydrogen arsenate (1:1,200)	1	0	1.55	.68	13	14	.1519	2.040	1:1.6660	1:.5000
Basic lead arsenate (1:50)	0	3	2.17	26.65	5	7	.3911	.486	1:2.3230	1:1.5100
Basic lead arsenate (1:50)	0	3	1.52	18.00	6	7	.4860	5.355	1:1.7650	1:1.2500
Basic lead arsenate (1:400)	1	.....	.34	.....	14	.....	.1139	.....	.....	.....
Calcium arsenate (2:50)	0	0	3.28	26.53	5	5	(a)	.....	.....	.....
Calcium arsenate (1:50)	.....	0	.....	12.56	.....	5	.....	.....	.....	.....
Calcium arsenate (1:400)	.....	1	.....	6.13	.....	5	.....	.....	.....	.....
Calcium arsenate (1:800)	.....	1	.....	1.17	.....	7	.....	.....	.....	.....

a Excrement not analyzed.

These results indicate that the lead hydrogen arsenate is the best spray for rapid effective killing, since a larger quantity of the poisonous element is assimilated by the caterpillar than either of the other types. Assuming that the lead hydrogen arsenate approximates closely an efficient insecticidal spray and calculating from the figure obtained as given in the above tables at the dilution 1 to 400, we may generalize as follows relative to the specific amount of arsenic pentoxid necessary to kill a certain number of caterpillars: It required approximately 0.1595 mgm. of arsenic pentoxid to kill 1,000 of the small tent caterpillars and 1.87 mgm. to kill a like number of the nearly mature larvæ. It is also interesting to note that, although a large percentage of the basic lead arsenate passes through the intestinal tract, the total amount of arsenic pentoxid devoured is about the same as for lead hydrogen arsenate—namely, 0.1139 mgm. for 1,000 caterpillars. It is supposed from the data, therefore, that it takes approximately the same quantity of arsenic, whether from lead hydrogen or basic arsenate, to kill equal numbers of caterpillars, but the amount varies greatly, depending upon the size of caterpillars used.

FIELD EXPERIMENTS WITH CALCIUM ARSENATE

Since but limited work has been done on the effects of calcium arsenate as a spray under climatic conditions existing in the Pacific Northwest, a preliminary field test was made in connection with the preceding

series of experiments to ascertain the burning tendencies of calcium arsenate upon foliage.

Two types of calcium arsenate were used: the calcium arsenate ($CaH.AsO_4$) described above and calcium ammonium arsenate, obtained by treating the former calcium arsenate with 2 per cent ammonium hydroxid, washing the resulting salt free of ammonia and drying at 110° C.

The analysis of the salt thus obtained is as follows:

	Per cent.
Calcium oxid (CaO), total	37.03
Arsenic pentoxid (As_2O_5), total	52.02

These two spray materials were tested out upon the foliage of young apple trees in the Oregon Experiment Station orchard. The trees were thoroughly and uniformly covered with the spray solutions. The suspension qualities of both materials were very poor, and they appeared on the foliage as coarse granules.

The different strengths of the spray as used were as follows:

No. 1. Calcium hydrogen arsenate (2:50), or 4.8 gm. in 1,000 c. c. of water.
No. 2. Calcium hydrogen arsenate (1:50), or 2.4 gm. in 1,000 c. c. of water.
No. 3. Calcium hydrogen arsenate (1:100), or 1.2 gm. in 1,000 c. c. of water.
No. 4. Calcium ammonium arsenate (2:50), or 4.8 gm. in 1,000 c. c. of water.
No. 5. Calcium ammonium arsenate (1:100), or 1.2 gm. in 1,000 c. c. of water.

The application was made on June 22, 1916. Showers were frequent the following two days, but, on examination of the foliage, material as coarse and granular as when applied was still evident. There was no indication of burn at this time. Frequent showers continued, and on June 28 the following effect was noted:

No. 1. Burn on leaves general, though scattered; spots of fair size.
No. 2. Burn on leaves general, though scattered; spots small.
No. 3. Burn slight, scattered; spots small.
No. 4. About same as No. 2.
No. 5. Burn about as general as No. 4; spots very small.

From this date on the burn spread slowly but steadily, and occasional showers continued. An examination on July 2 showed the burn to be spreading and in all cases was too severe for practical use.

From these preliminary tests and under the climatic conditions noted, calcium arsenates can not, at present, be recommended as a safe spray material.

SUMMARY

(1) Lead hydrogen arsenate has a higher killing efficiency at a given dilution than either calcium or basic lead arsenate.

(2) It requires a longer period of time to kill the nearly mature caterpillars than the small forms.

(3) All of the arsenic devoured by the insects in feeding upon sprayed foliage is not assimilated, but a portion passes through the intestinal tract in the excrement. The percentage amount of the arsenic assimilated depends upon the arsenate used; lead hydrogen arsenate was assimilated readily and most of the arsenic was retained in the tissue, while much of the basic lead arsenate was found in the excrement.

(4) It requires approximately 0.1595 mgm. of arsenic pentoxid to kill 1,000 small tent caterpillars, and approximately 1.84 mgm. of arsenic pentoxid to kill 1,000 nearly mature tent caterpillars, irrespective of the particular arsenate used as a spray.

(5) Preliminary experiments on the burning effects of calcium arsenate indicate too severe injury to warrant the practical use of this spray.

ADDITIONAL COPIES

OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT

30 CENTS PER COPY

SUBSCRIPTION PRICE, $3.00 PER YEAR